A COMPARISON OF THE MODE OF ACTION OF ANGIOTENSIN AND CERTAIN OTHER NATURALLY OCCURING POLYPEPTIDES

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Thesis submitted for the degree of

Doctor of Philosophy

of the University of Edinburgh

in the Medical Faculty

July 1967

CONTENTS

The cardiovascular systems of animals are affected in different ways by the three naturally occuring polypeptides angiotensin, oxytocin and vasopressin. Molecule for molecule angiotensin is the most potent vasoconstrictor known, and causes a contraction of the vascular smooth muscle in all the species studied. The vascular actions of oxytocin and vasopressin are somewhat more variable among different species and in a given species, it may on occasion produce either a vasoconstiction or a vasodilatation or be without any apparent effect: That at least part of this variability in the vascular action of oxytocin and vasopressin is linked with the circulating levels of ovarian hormones and/or the prevailing degree of activity in the sympathetic nervous system has been established over the past decade or so by the work of Pickford and her co-workers. In the case of angiotensin too there is a great deal of evidence that its action on smooth muscle is in some way inter-connected with that of the sympathetic nerve supply to the part. It would be contrary to what one expects of the economy of biological engineering to find that each of these three polypeptides initiates a unique and independent chain of events in the vascular smooth muscle leading to its contraction or relaxation. It is far more likely that a link in a common chain is affected by each vasoactive substance. This work was, therefore, an effort to see whether those factors that had been shown to affect the vascular actions of oxytocin and vasopressin had any influence on the actions of angiotensin on the cariovascular system. It is always hoped that this would give a better understanding of the actions of these polypeptides and some further insight into the fundamental processes involved in the activation of vascular smooth muscle.

CHAPTER 1

FACTORS MODIFYING THE ACTIONS OF THE POLYPEPTIDES

VASOPRESSIN

1. Effect of ovarian hormones.

Byrom (1938) showed that a dose of vasopressin which did not have any effect on immature rats could be made to produce extensive renal infarction by the prior treatment of such animals with gonadotrophins. Since ovariectomy protected these animals from the sensitising effect of gonadotrophins and oestrin administration had a similar sensitising effect he concluded that oestrogens were responsible for enhancing the response of the vascular system to vasopressin. Lloyd (1959a) found that rats in oestrus showed a greater response to vasopressin than dioestrous females. Following ovariectomy, the dose of vasopressin required to give a constriction of the vessels of the mesoappendix as observed in a transparent chamber, or the dose required to give a standard elevation in the blood pressure, increased after the third post operative day (Lloyd 1959a). However, for some unknown reason the sensitivity to vasopressin returned to normal on about the seventh post-operative day. During the period of reduced sensitivity administration of oestrogen and progesterone to these animals restored the sensitivity to normal. Honore and Lloyd (1961) showed that male rats gave a smaller pressor response than female rats of comparable weight to a given dose of vasopressin. Following castration male rats were shown to be more sensitive to the pressor action of vasopressin, the opposite state to that seen in ovariectomised females. After the 10th day of pregnancy the pressor response to vasopressin was again found to be enhanced compared with that of the dioestrous rat (Lloyd 1959b). An enhancement of the pressor response to vasopressin in pregnant rats has also been reported by Ostrovsky and Gornall (1964). In these instances however, either chorionic gonadotrophins or the

ovarian hormones can be incriminated.

The evidence for a similar sensitising effect of oestrogens in the case of man are not entirely concordant. Reynolds (1952) found that humans treated with oestrogens were more sensitive to the pressor action of posterior pituitary extract. He does not appear to have repeated these experiments using more purified preparations or synthetic vasopressin. Browne (1964) showed that pregnancy increases the systolic pressure rise to an intravenous injection of pituitrin. However he concluded that the sensitising agent was chorionic gonadotrophins. On the other hand, de Vallera and Keller (1938) and Schockaert and Lambillon (1937) find that pregnant women are less sensitive to vasopressin.

2. Effect of the sympathetic nervous system.

Kepinow (1912) and Niculescu (1914) had reported that pituitrin enhances the response of blood vessels to adrenaline. Thompson (1938) has confirmed this both in the intact animal and isolated blood vessels and has found the enhancement to persist even after constrictor action of vasopressin has passed off. Melville and Stehle (1931), however, failed to confirm these early findings. Wagner and Braunwald (1956) studied three subjects showing an autonomic deficiency syndrome and found that they showed a pressor response to very small doses of vasopressin, whereas, much larger doses were without effect on normal subjects. This extreme sensitivity to vasopressin cannot be attributed solely to a lack of sympathetic nervous activity, for these subjects also showed interference with the parasympathetic system as indicated by the presence of impotence. These workers also demonstrated that a similar sensitivity to the action of vasopressin could be induced in normal subjects by the prior treatment with tetraethyl ammonium chloride (T.E.A.C.) This fact again though suggestive of participation of the

sympathetic nervous system in this effect, may be criticised on the grounds that T.E.A.C. has been shown to have a similar action on several other vasoconstrictors, suggesting some direct action of this substance on the vascular smooth muscle in addition to a ganglion blocking action (Lum&Rashleigh 1961; Page and Taylor 1950). In rats, pithing, decerebration, ganglion block with hexamethonium and tetraethyl ammonium iodide, or peripheral sympathetic block using reserpine, bretylium, or dihydroergotamine were all found to increase the pressor response to vasopressin (Lloyd and Pickford 1961). Atropine however did not affect the response to vasopressin. Similarly Supek et all (1962) found that in the anaesthetised dog hydergine, dibenamine and tolasoline increased the pressor response to vasopressin. On the other hand Youmans and Rankin (1947) did not find any influence of dibenamine on the response to vasopressin in the anaesthetised dog: and Meier, Tripod and Studer (1958) using the perfused hind limb of the rabbit found that dihydroergotamine and hydergine decreased the response to vasopressin slightly though Regitine enhanced it. The effect of these drugs is probably due mostly to the abolition of compensatory reflex mechanisms.

Another possible site for the interaction of vasopressin with the autonomic nervous system is suggested by the work of McCubbin, Page and Bumpus (1957) who showed that vasopressin like noradrenaline could diminish the carotid sinus occlusion reflex when applied locally to the carotid sinus. Noradrenaline was much more effective at this site than vasopressin and they probably act by altering the state of contraction, and therefore the resistance to stretch, of the baroreceptive arterial wall thus modulating the sensitivity of the receptor to intra-arterial pressure changes. Kezdi (1954) has suggested that this mechanism of action of drugs may play an important physiological role.

OXYTOCIN

1. Effect of ovarian hormones.

The vascular action of oxytocin in the mammal seems to have both vasoconstrictor and vasodilator components. Thus Gaddum (1928) using the separated active principle of the posterior pituitary found that the oxytocic fraction produced a depressor response in cats when the vascular tone was high after the administration of vasopressin. This effect was not obtained in all the preparations, while vasopressin always produced a pressor response. Van Dyke, Adamsons and Engle (1955) showed that in the anaesthetised rat large doses of oxytocin (300mU) had a blood pressure elevating effect equivalent to about 4mU of vasopressin while smaller doses caused a fall in the blood pressure. Using changes in the pulse contour to detect vasodilatation Woodbury at al (1944) found evidence of vasodilatation in three human subjects which they studied, on the administration of pitocin. They concluded however that the fall in the blood pressure which they observed was mainly due to a weakening of the action of the heart. In a latter study using cats, rabbits and dogs, Woodbury and Abreu (1944) detected a vasodilation in the cats and rabbits though not in the normal dogs. Lloyd (1959a) has shown that in male and dioestrus female rats 20 to 100mU of synthetic oxytocin produces a vasodilatation of the vessels in the mesoappendix even though the blood pressure was unaffected. A similar vasodilatation has been demonstrated in the hind limb of both male and female dogs (Lloyd and Pickford 1962). In man too intravenous injections or intravenous infusions of oxytocin into males or non pregnant females caused a fall in the blood pressure and a vasodilatation in the skin and muscle vessels (Mayes and Shearman 1956; Kitchin, Lloyd and Pickford 1959). Thus the major action of oxytocin in all these reports is one of vasodilatation.

Lloyd (1959 a and b) first showed that unlike dioestrous rats those in natural oestrous responded to oxytocin by a rise in blood pressure which accompanied a constriction of the vessels of the mesentery. Oestrogen administration to dioestrous or male rats converted their response to oxytocin from one of vasodilatation to vasoconstriction as observed in the meseneteric vessels and this was accompanied by a rise in the blood pressure. In the case of female animals the conversion of the reaction to oxytocin could be achieved by treatment with either stilboestrol (3.5µg/100g body weight) or progesterone (90µg/100g body weight) given 24 hours previously; while male animals required stilboestrol or stilboestrol and progesterone. Progesterone alone was inadequate in the case of males. That progesterone is as effective as oestrogens in the female rat in bringing about the conversion in vascular response to oxytocin has been confirmed by Fullerton and Morrison (1965). Ovariectomy lead to a temporary sensitisation to the dilator action of oxytocin, and the treatment of these animals with stilboestrol and progesterone in combination restored the oestrous type of constrictor response. Rats from the tenth day of pregnancy to the third day post partum also responded by an elevation in the blood pressure. Since this was observed even when the uterus was excluded from the circulation it was not secondary to an expulsion of blood from a uterine contraction induced by oxytocin. Fullerton and Morrison (1965) have observed similar results in pseudopregnant rats.

In the dog too similar observations have been made (Lloyd and Pickford 1962). Thus intravenous or intra-arterial oxytocin was found to produce a vasodilatation in the hind limb vessels of both normal male and female dogs; the blood pressure remained unchanged. Pre-treatment of these animals with either oestrone or stilboestrol leads to the conversion of this response to

a constrictor one. This conversion takes place in as short a time as 30 mins. after the intravenous administration of an oestrogen. The same phenomenon has been described in the hand blood flow of man 40 mins. after oestrogen was injected into a brachial artery (Haigh, Kitchin and Pickford 1963). As already mentioned, studies on men and non-pregnant women have shown that oxytocin produces a vasodilatation in them. In contrast however there are several reports that a proportion of pregnant women respond to oxytocin by elevation in the blood pressure. This again suggests a basic similarity in the vascular response to oxytocin in the rat and man.

There are three recent reports however that do not appear to agree with these findings. Nakano (1964) found that both male and pregnant rats responded to synthetic oxytocin by an elevation in the blood pressure which was dose dependent in the range of 0.05 to 3.2 units per Kg body wt. In male dogs that were pretreated with diethylstilboestrol (0.25mg/kg for 3 days) as well as in pregnant dogs., Nakano and Fisher (1963) found that oxytocin increased the flow in the coronary, common carotid, brachial, femoral, & superior mesenteric arteries when administered in doses ranging from 0.005 to 0.05u/kg. Katz (1964) has shown that in cats pure synthetic oxytocin in doses of 1 to 20 units/kg always produced an elevation in the blood pressure. They attributed the fall in the pressure observed on occasions with the commercial preparation of Syntocinon, to a depressor effect of the preservative substance in this preparation, viz chlorbutanol. It is however obvious that all these workers have used very large doses of oxytocin well outside the doses used by Pickford et al, and the results are therefore not comparable.

2. Effect of the sympathetic nervous system.

Wagner and Brundwald (1956) showed that an infusion of 10 to 20 units

of oxytocin per hour into their subjects suffering from the autonomic deficiency syndrome produced a brief fall in the pressure followed by a prolonged rise whereas normal subjects did not show any response to the same dose. Interference with the autonomic nervous system by pithing, decerebration, dihydroergotamine, bretylium,dibenamine, reserpine or tetraethyl ammonium treatment all caused oxytocin to become pressor (Lloyd and Pickford 1961). Since in this study other depressor substances such as isoprenaline or acetylcholine were found not to affect the response to oxytocin it is certain that the reversal of the action of oxytocin was not related to a reduction in the vascular tone per se. It is worthy of note that following reserpine treatment an infusion of noradrenaline failed to restore the dilator action of oxytocin although the response to tyramine was increased by this procedure. A similar reversal in the response of the hind limb vessels of the dog to oxytocin has been observed after acute sympathectomy or treatment with bretylium, hexamethonium or dihydroegotamine (Lloyd and Pickford 1962). In these experiments, the stimulation of the peripheral end of the cut lumbar sympathetic chain was found to restore the dilator response to oxytocin (Haigh, Lloyd and Pickford 1964). In dogs with chronic unilateral lumbar sympathectomy the response of the vessels of the denervated limb to oxytocin was a vasoconstriction for as long a period as re-innervation was demonstrably absent (Haigh, Lloyd and Pickford 1965). Deis, Kitchin and Pickford (1963) have shown that the effect of oxytocin on the hand blood flow in man can be similarly reversed by blocking the nerves to the limb with lignocaine, or by intra-arterial bretylium.

The effect of some of the procedures that reverse the vascular response to oxytocin on the extracellular cations has also been investigated because changes in the extracellular concentration of ions are known to influence

the action of drugs on vascular smooth muscle. No correlation was found between the cation concentration and the procedures affecting the response to oxytocin (Pickford and Lloyd 1966). Induced changes in the extracellular ion composition as well as drugs that can be expected to modify the ionic equilibria across the cell membrane were similarly found to be without effect on the response to oxytocin.

Thus it is clear that the administration of oestrogen and progesterone or interference with the sympathetic nervous system influences the vascular actions of vasopressin and oxytocin in the same direction. As pointed out earlier, exogenous noradrenaline fails to restore the dilator response to oxytocin following sympathectomy even though stimulation of the peripheral end of the cut sympathetic chain is effective in this respect. It has been found, however, that after sympathectomy an infusion of adrenaline can restore the dilator response to oxytocin. In the situation where oestrogens have converted the oxytocin response to a constrictor one in the dog, the intra-arterial infusion of adrenaline alone greatly reduces the constrictor response to oxytocin; while if atorpine as well as adrenaline is present the dilator response to oxytocin is re-established. Similarly in the presence of atropine sympathetic nerve stimulation is effective in restoring the dilator response to oxytocin in the oestrogen treated dog or monkey (Haigh, Lloyd and Pickford 1965). Fullerton and Morrison (1965) have shown that in the oestrogen treated rat too, stimulation of the central sympathetics by using eserine (Varagic 1955; Varagic et all 1961) abolished the vasoconstrictor response to oxytocin. The additional presence of atropine did not affect the result in any way in this species. It was also found that eserine failed to influence the response to oxytocin in progesterone treated rats, and in this respect the effect of oestrogens differed from progesterone

in this species.

In any of these situations an alpha receptor blocking agent will at once convert the oxytocin response to one of constriction. Thus adrenaline infused in the presence of dihydroergotamine does not restore the dilator effect of oxytocin (Lloyd and Pickford 1966). This suggests that the beta receptors are not concerned with these effects, and this is confirmed by the inability of nethalide to influence either the constrictor or dilator effects of oxytocin. On the other hand the importance of the alpha receptor is shown by the fact that an infusion of adrenaline prevents the constrictor response to oxytocin after hexamethonium.

On the basis of this evidence it has been suggested that the fraction of adrenaline released by sympathetic nerves is of functional significance, and is essential in some way for the dilator effect of oxytocin; and that oestrogens interfere with the manufacture of adrenaline or its release from the sympathetic nerves. Oestrogens have been shown to increase the acetylcholine content of a variety of organs (Reynolds and Foster 1940; Reynolds 1939). The necessity for the presence of atropine for the restoration of vasodilatation after oestrogen conversion in contrast to the sympathectomy is probably linked in some way with this.

ANGIOTENSIN

1. Effect of the ovarian hormones.

The evidence for an effect of oestrogens on the vascular actions of angiotensin is of an indirect nature. Harrison, Grollman and Williams (1940) noticed that in rats with renal hypertension the blood pressure decreased spontaneously during the last few days of pregnancy and returned to the previous high level after delivery. They also showed that pregnant rats were less sensitive to the pressor action of renin. These results were confirmed

by Page, Patton & Ogden (1941) who also found that pseudo-pregnancy in the rat had a similar effect. It is possible to attribute these changes to the altered hormonal balance of the animal during pregnancy, on the other hand they may be due to some other change brought about by the state of pregnancy. Further it is by no means established that the pressure elevation in renal hypertension is due to the excessive endogenous production of angiotensin. Mackaness and Dodson (1957) found a reduction in the pressor response to renin in the pregnant rat, but Dodson (1957) was unable to find any change in the response to natural angiotensin. Ostrovsky and Gornall (1964) however using synthetic angiotensin have demonstrated a significant reduction in the response in the last week of gestation. Mackaness (1959) has shown that there is a significant reduction in the level of angiotensinogen in the pregnant animal, and attributed the reduced response to renin to this. Page (1947) on the other hand found that puerperal women needed higher doses of angiotensin to produce a given elevation in the blood pressure when compared with non-pregnant women, and concluded that this was due to an increase in the level of angiotensinase. Chesley (1963) too has found that pregnant women are less responsive to angiotensin. Abdul-Karim and Assali (1961) showed that both the height and the duration of the pressor response to angiotensin was reduced during pregnancy and that the smallest responses were observed in women near term. The latter finding suggests that the change in the response to angiotensin was not directly related to the increase in the blood volume as this usually reaches a maximum in the seventh and eighth month of gestation and decreases towards term.

The only study of a direct action of oestrogens on the response to angiotensin appears to be that of Page and Helmer (1940) who found no change in the pressor effect following stilboestrol administration.

2. Effect of the sympathetic nervous system.

There is evidence that angiotensin may interact with the autonomic

nervous system at more than one point.

Von Euler and Sjöstrand (1941) showed that in cats and rabbits decerebration at a level below the posterior corpora quadrigemina reduced the pressor response to natural angiotensin, while transection above the superior corpora leaves the action of angiotensin unaltered or even enhanced. That these effects were not secondary to changes in general vascular tone were shown in experiments where parallel reductions in the blood pressure were produced by haemorrhage or manipulation of the viscera. Bickerton and Buckley (1961) cross circulated a dog's head with blood from a donor animal and showed that the injection of 0.2 to 0.4µg of angiotensin into the head circulation of the recipient caused a pressor effect in its general circulation which was abolished by pre-treatment with piperoxan or besodioxan. The pressor effect of an intravenous injection of angiotensin into the recipient animal was unaffected by these two drugs. Denervation of the carotid sinus left the effect of angiotensin in the head circulation unaltered. A brief occlusion of the circulation to the head to mimic a period of anoxia, as may be caused by a cerebreal vasoconstrictor action of angiotensin, also failed to produce a response in the recipient animal. Any possible leak between the head circuit and the recipients general circulation was excluded by injection of I^{131} into one circuit and analysing blood from the other. This then is strong evidence for a central action of angiotensin. More recently Buckley (1965) has shown that urinary adrenaline but not nor-adrenaline excretion is increased in the recipient during the introduction of angiotensin into the head circuit. Benetato et al (1963) have confirmed these findings and also made the interesting observation that atropine or reserpine treatment (given 12 hours before) abolished these central actions of angiotensin, and that in reserpine treated animals

the central effect can be restored by an infusion of noradrenaline into the cerebral circulation. This suggests the presence of a cholinergic as well as a adrenergic link in the central mechanism activated by angiotensin. Similarly in cross circulation experiments Laverty (1963) has found that angiotensin intravenously, in large doses, caused a vasoconstriction in the vascularly isolated but inervated hind limb of the rat. Smaller doses of angiotensin I.V. produced a vasodilatation in this preparation as did noradrenaline. That these were nervously mediated changes was confirmed by the fact that they were abolished by section of the nerves. Dickinson and Lawrence (1963) have shown that an infusion of small quantities of angiotensin, insufficient to produce a direct pressor action (0.005 to 0.0075 µg/kg/Min.,) into conscious rabbits over a period of days, leads to a gradual elevation of their blood pressure. Since this elevation in the blood pressure can be restored to the pre-infusion basal level by the administration of the short-acting ganglion blocking drug trimetaphan (Arfornad) it is likely to be due to an increase in the activity of the sympathetic nerves (Yu and Dickinson 1965). On the grounds that such an infusion of angiotensin when made into a vertebral artery was more effective than an intravenous one, these workers suggest that the increased sympathetic nerve activity is secondary to a vasoconstriction produced in the region of the medullary vasomotor centre. These findings can however be equally interpreted as being due to a direct action of angiotensin on the nerve centres, specially as Mandel and Sapirstein (1962) have shown that sub-pressor doses of angiotensin increase rather than decrease the cerebral blood flow. The infusion of sub-pressor quantities of angiotensin over long periods have been shown to produce similar elevations of the blood pressure in the dog (McCubbin et al 1965) rat (Koletsky, Rivera-Velez and Pritchard 1965)

and man (Laragh, Cannon and Ames 1964).

Using an ingenious technique Berry, Austen and Clark (1964) divided the circulation of anaesthetised dogs into a cardiopulmonary circuit and a systemic circuit. The only possible connection between the two circuits was through any collaterals of the bronchial circulation. In this preparation when angiotensin was injected into the systemic circuit it produced a positive inotropic and chronotropic effect while when it was injected into the cardiopulmonary circuit so that it could reach the heart directly it had the opposite effect. The positive inotropic and chronotropic effects were abolished by the beta receptor blocking drug nethalide. The positive inotropic and chronotropic effects when angiotensin is introduced into the systemic circuit is therefore probably a central action of angiotensin.

Gregory, Levine and Lindley (1944) however found that angiotensin caused equal rises in pressure before and after spinal anaesthesia in both hypertensive and normotensive human subjects. In dogs too they found that vagotomy and low section of the spinal cord did not affect the response to angiotensin. Against this negative finding in man is the report of Scroop and Whelan (1966) that an intravenous infusion of angiotensin produced a vasoconstriction in the hand and that this was greatly reduced by the prior administration of phenoxybenzamine or bretylium. This response was found to be absent in surgically sympathectomised or nerve blocked hands. This clearly points to a central action in man as well but whether this action is on the vasomotor centre or some other site has not been established.

Another possible site at which angiotensin and the autonomic nervous system may interact is at the ganglion, or at its equivalent the adrenal medullary cell.

Braun Menendez et al (1940) observed that the injection of angiotensin

caused the discharge of adrenaline from the denervated adrenals. Renson, Barac and Bacq (1959) noticed that after treatment with cocaine, angiotensin caused a contraction of the nictitating membrane in the cat and that this effect was blocked by phenoxybenzamine. Feldberg and Lewis (1964) demonstrated that in the eviscerated cat the injection of angiotensin into the coeliac artery so that it reached the adrenals produced a release of catecholamines as shown by the contraction of the denervated nictitating membrane of the animal. In a sensitive preparation as little as 1ng intra-arterially could be shown to cause the release of catecholamines. 0.1 to 0.2 µg of angiotensin intravenously had a similar effect, and adrenalectomy abolished these effects. The simultaneous changes in the blood pressure suggested that the main catecholamines released in this way was adrenaline. Other vasoconstrictors were ineffective in this respect while the vasodilator bradykinin could also liberate catecholamines from the adrenals. This latter finding is against the suggestion made by Kaneko et al (1961) that the release of adrenaline which they had observed earlier with the intra-arterial injection of a number of vasoconstrictors including angiotensin, after a sensitising dose of DMPP is due to the local tissue hypoxia from local vasoconstriction. Vogt (1965) has confirmed Feldberg and Lewis' findings using isolated perfused adrenals and estimation of the released catecholamines by chemical means. Feldberg and Lewis (1965) have subsequently shown that chronic bilateral splanchnic nerve section or the administration of hexametonium which reduces the sensitivity of the medulla to acetylcholine, were both without effect on the sensitivity of the adrenal medulla to angiotensin. There would therefore appear to be different receptors on the medullary cells for acetylcholine and angiotensin.

Lewis and Reit (1965) demonstrated that the close intra-arterial

injection of angiotensin into the superior cervical ganglion of the cat caused a contraction of the nictitating membrane. The smallest effective dose of angiotensin in this respect was 0.1µg which is comparable to the dose of other naturally occuring substances required to excite the ganglion. Hexamethonium was without any influence on this action but removal of the ganglion or sectioning of the post-ganglionic nerves abolished the response. Tachyphylaxis developed to the action of angiotensin but when this had occured acetylcholine was still effective in exciting the ganglion. Chronic decentralisation of the ganglion lead to sensitisation to the stimulating action of angiotensin. These workers therefore came to the conclusion that angiotensin exerts a ganglion stimulating action by combining with receptors different from those concerned with acetylcholine induced stimulation. These results have been confirmed by Panisset,Biron and Beaulnes (1966) who found in addition that the stimulating effect of smaller doses of angiotensin on the ganglion could be blocked by atropine and hexamethonium though larger doses were not influenced by these drugs. Like Lewis and Reit they found that when the preganglionic nerve was stimulated submaximally retrograde injection of angiotensin towards the ganglion in a dose of 0.01 to 0.1ng resulted in an enhancement in the response to subsequent stimulation. In smaller doses however (0.001-0.01ng) angiotensin had a inhibitory effect on preganglionic nerve stimulation. It is interesting that this inhibitory action of angiotensin was prevented by dihydroergotamine - a finding that could implicate noradrenaline in the synaptic effect of angiotensin. That noradrenaline can have an inhibitory action at the synapse was shown by Marrazzi (1939). Such a dual action of angiotensin at the ganglion is in agreement with the observation of Haefely, Hurlimann and Thoenen (1965).

Further evidence for an effect of angiotensin on ganglia comes from observations of its action on the gut. Robertson and Rubin (1962) demonstrated that in the rabbit and guinea-pig ileum, angiotensin induced contractions as well as contractions due to acetylcholine and nicotine, could be potentiated reversibly by the anti-cholinesterase drug BW284C51. Atropine at doses that reduced the response to nicotine also reduced the response to angiotensin. Botulinum toxin was found to abolish the response to nicotine and angiotensin while being without effect on the response of this preparation to acetylcholine. The conclusion that in this preparation too, a large part of the stimulating action of angiotensin is due to an action on the ganglia in the wall of the gut is therefore inescapable. Since in this preparation hexamethonium was without effect on the angiotensin response but abolished the response due to nicotine it suggests that in this tissue as well, angiotensin is acting on non-acetylcholine receptors on the ganglion cell.

In the isolated guinea-pig vas deferens angiotensin strongly potentiates the response to electrical stimulation of the hypogastric nerve, although angiotensin has no direct effect on the vas (Benelli, Della Bella and Gandini 1964). Noradrenaline and acetylcholine, both of which contract the vas, were not similarly affected by angiotensin. Although the hypogastric nerve was originally described as a postganglionic sympathetic fibre, it is now certain that ganglionic cells are interposed (Sæjostrand 1962; Merrillees, Burnstock and Holman 1963). It is therefore likely that in this situation too angiotensin is acting at a ganglionic site, probably playing a permissive role in the transmission through the ganglion. However, the pharmacology of the vas deferens - hypogastric nerve preparation must still be regarded as problematical firstly since some reports suggest that the nerve is a mixed one (Della Bella, Gandini and Preti 1964) with both sympathetic and parasympathetic efferent pathways, secondly since treatment with anti-nerve

growth factor leaves the hypogastric nerve ganglia unaffected, and thirdly since it has been shown that adrenegic blocking agents modify only slightly the effect of electrical stimulation while abolishing the response to exogenous noradrenaline (Della Bella, Gandinimand Preti 1964; Boyd, Chang and Rand 1960).

Benelli, Della Bella and Gandini (1964) have also shown that in anaesthetised or spinal cats a depolarising ganglion block with nicotine or tetraethyl ammonium diminished the pressor response to intravenous angiotensin; but if the dose of nicotine is increased so that the block becomes of the competitive type the pressor response to intravenous angiotensin is greatly enhanced. This enhancement was abolished by treatment with hexamethonium. Trendelenberg (1961) has shown that when histamine and pilocarpine were acting as ganglion stimulating drugs, competitive ganglion block with nicotine produced an enhancement of the response to these drugs and that hexamethonium prevented this enhancement. By analogy with these two drugs therefore, angiotensin would appear to be acting as a ganglion stimulator in this instance too.

McGiff and Itskovitz (1964) has demonstrated, that in the dog, vasoconstriction in the renal vascular bed on intravenous injection of angiotensin into the animal could be abolished by renal denervation while direct injection into the renal artery was still effective in producing a vasoconstriction. McGiff and Fasy (1965) subsequently showed that bretylium, guanethadine or reserpine treatment was also effective in abolishing the renal vasoconstriction due to intravenous angiotensin, while pentolinium and hexamethonium were ineffective. They further observed that a parallelism existed between the action of nicotine and angiotensin on the renal vasculature. Thus when nicotine had lost its vasoconstrictor effect after repeated injection,

angiotensin too was found to have lost its effect; and injection of nicotine reduced the response to an intravenous injection of angiotensin given subsequently. These results again appear to point to a ganglionic site of action of angiotensin.

A mode of action at the ganglion completely different from that described so far has very recently been suggested by Panisset (1967). He has shown that angiotensin in a dose of 0.1 to 100ng increased the acetylcholine output from the perfused superior cervical ganglion. In larger doses however the acetylcholine output was inhibited. Antiotensin was similarly found to increase the output of acetylcholine from electrically stimulated guinea-pig ileum. This would suggest that angiotensin is acting at the pre-synaptic membrane, or as an anticholinesterase.

Perhaps the first to show that angiotensin may interact with the sympathetic nervous system at a peripheral site as well was Mylon and Heller (1948). They showed that in the isolated rabbit ear perfused with physiological saline natural angiotensin alone was not a constrictor, but when combined with traces of adrenaline, which by itself were not pressor, produced a strong vasoconstriction. They came to the conclusion that the tyrosine contained in the molecule of angiotensin was of importance for this action since the amino-acid tyrosine in combination with adrenaline gave a powerful constriction. It is evident however that their preparation of angiotensin was not pure as it produced a vasolilatation when given by itself. Using the same preparation Sakurai and Hashimoto (1965) have shown that synthetic angiotensin greatly potentiated the response to noradrenaline and tyramine.

McCubbin and Page (1963) demonstrated that the infusion of angiotensin into anaesthetised dogs increased the vascular responses to tyramine

ephidrine, and DMPP. Since there is evidence to show that tyramine and ephidrine release catecholamines stores and DMPP can be expected to do the same through its ganglion stimulating action they suggested that angiotensin either sensitises the release of noradrenaline or sensitises the vascular receptor to nor-adrenaline. The latter possibility is rendered unlikely as they could find no change in the pressor action to injected noradrenaline. It is worth noting that this sensitising action of angiotensin persisted even after tachyphylaxis to its pressor action had been induced. Interference with the sympathetics by ganglion block, spinal cord section or bretylium treatment immediately abolished the sensitising effect of angiotensin on the response to tyramine or ephidrine. Using a constant flow perfusion technique they also showed a similar sensitising action of angiotensin on the responses of the vascular bed supplied by the superior mesenteric artery of the dog to intra-arterial tyramine. On the other hand they failed to demonstrate a similar effect in the hind limb circulation. On the basis of these findings McCubbin and Page postulate that angiotensin increases the amount of endogenous noradrenaline released in response to a given stimulus. However Buckley (1965) has failed to find any effect of angiotensin on the response of the isolated perfused rabbit kidney to tyramine.

Zimmerman (1962) studied the effects of angiotensin on the hind limb vessels of the dog by introducing a constant flow pump between the cut ends of the aorta so that changes in the vascular resistance induced by drugs introduced intra-arterially gave rise to proportionate changes in the perfusion pressure. He found that ganglion block using hexamethonium or surgical sympathectomy at the level of lumbar 5 reduced the responses to angiotensin while leaving the responses to noradrenaline and tyramine unaffected. Stimulation of the peripheral end of the cut sympathetic trunk partially restored the response

to angiotensin, but an infusion of nor-adrenaline was ineffective in this respect. Cervical spinal cord section was found to have an effect similar to lumbar sympathectomy. He therefore concluded that the normally functioning sympathetic nerves were capable of facilitating the response to angiotensin although a more precise mechanism for this effect was not apparent from his experiments. Baum's (1963) results, using the same experimental arrangement confirm that acute sympathectomy reduced the response of the hind limb vessels of the dog to angiotensin, and have shown in addition that reserpine pre-treatment (0.2 mg/kg i.m. 48 hrs. before) can produce a similar result.

Several reports also suggest that the effects of angiotensin on the peripheral sympathetics may be to cause a release of noradrenaline from the nerve terminals. Krasney et al (1965) using anaesthetised dogs with denervated baroreceptors found that angiotensin had a positive inotropic and chronotropic effect which could not be abolished by spinal cord section at cervical six, bilateral removal of the sympathetic chain or ganglion block. Bretylium, however, abolished and beta receptor blocking drugs greatly reduced this effect of angiotensin on the heart thus suggesting an influence on adrenergic nerve endings. Beaulnes (1963) found that the development of tachyphylaxis to the inotropic and chronotropic action of angiotensin on isolated atria can be prevented by noradrenaline and that dichlorisoproterenol partially blocks the action of angiotensin on this preparation, suggesting again a peripheral site of action of angiotensin on the cardiac sympathetics. Downing and Sonnenblick (1963) observed a persistent depression of the ventricular function curve of dogs' hearts after an infusion of angiotensin and this could not be attributed to a spontaneous deterioration of the preparation. They therefore suggested the possibility that it was due to a chatecholamine depletion which had occured

during the angiotensin infusion. Distler, Liebau and Wolff (1965) have shown that mammalian arterial strips lose 40-60% more of their noradrenaline content when washed with Tyrode containing angiotensin as compared with strips washed in plain Tyrodes solution. When rat aortic strips were made to contract by repeated applications of angiotensin the contractions grew weaker in an exponential manner; rinsing the preparation in noradrenaline solution (10^{-6} g/ml) for 10 mins. restored the response to angiotensin, and cocaine was found to prevent this restoration by noradrenaline. Feldberg and Lewis (1965) demonstrated that large intravenous doses of angiotensin produce a delayed contraction of the nictatating membrane in adrenalectomised cats. They concluded that this must be due to the release of catecholamines from the adrenegic nerves or extra-medullary chromaffin tissue. However, Muscholl and Vogt (1964) could not demonstrate that angiotensin released catecholamines from extra-medullary chromaffin tissue in the dog though nicotine was quite effective in this respect.

Earlier Benelli, Della Bella and Gandini (1964) had found that in the isolated perfused cat spleen preparation, angiotensin in doses too small to cause a direct contraction, greatly potentiated the splenic contraction induced by nerve stimulation. Two recent attempts have been made to elucidate the mechanism of this potentiation. Hertting and Suko (1966) using a constant pressure perfusion technique confirmed that angiotensin potentiated the response of the spleen to nerve stimulation. They were however unable to find any definite difference in the total catecholamine output during periods of nerve stimulation in the presence of absence of angiotensin. On the other hand a vasoconstriction produced by vasopressin or stopping the perfusion prior to nerve stimulation produced a potentiation of the splenic volume change in the same way as did angiotensin. They concluded therefore that

angiotensin acted by producing a vasoconstriction in the spleen thereby increasing locally the concentration of the noradrenaline liberated from the nerve terminals. They also showed that after chronic denervation or reserpine treatment,when the noradrenaline content of the spleen was less than 0.02µg/gm and tyramine was rendered inactive, angiotensin still produced a contraction of the spleen as well as a vasoconstriction. Since, however, they used a constant pressure prefusion technique which allowed changes in the rate of flow of the perfusate to occur, and since the size of the spleen as well as the amount of noradrenaline leaving the spleen can be affected by this in a complicated way, the interpretation of their results are not entirely certain. Thoenen, Hurlimann and Haefely (1965) using the more acceptable constant flow perfusion technique have again confirmed that angiotensin potentiates both the increase in the vascular resistance and the change in splenic volume produced by nerve stimulation. The change in the vascular resistance was potentiated more (70%) than the splenic contraction (20-30%). The noradrenaline output was however not measurably altered by the presence of angiotensin. Nor could angiotensin be shown to influence the uptake of noradrenaline infused into the spleen. These workers therefore came to the conclusion that the site of the enhancing action of angiotensin lies at the level of the smooth muscle cell. Against the failure to show an interference of the re-uptake of noradrenaline in these two studies is the recent report of Palaic and Khairallah (1967). They have shown that at an angiotensin concentration of 20µg/ml in the incubating medium noradrenaline uptake is inhibited in spleen and brain slices to the extent of 50%, while in rat aortae a 30% decrease in uptake was observed. These workers state that the high concentration of angiotensin in the medium was rendered necessary because of the powerful angiotensin destroying properties

of the tissues.

Against a catecholamine releasing role for angiotensin are the findings of Buckley (1965) who could detect no change in the catecholamine content in rabbit kidneys which were perfused with angiotensin for one hour. Similarly hearts and kidneys of rats receiving 1.0µg/kg/min. of angiotensin intravenously for one hour failed to show a change in their catecholamine contents. However in such an experiment the catecholamine releasing effect of angiotensin on the adrenal medulla would have to be taken into account. More direct evidence for an effect of angiotensin on the catecholamine content of nerve terminals comes from the electron microscopic studies of panagiotis and Hungerford (1966). They have shown that the subcutaneous administration of 6ug of angiotensin per day for 12 days into rats can significantly increase the number of granulated vesicles in the nerve terminals of the pineal glands. The pineal gland is known to be richly supplied with sympathetic nerve endings and earlier work has demonstrated that these granules contain catecholamines.

CHAPTER 3.

DEFINITION OF THE AREA REQUIRING INQUIRY

It is clear from the preceeding survey of the literature that oestrogen and progesterone exert a marked effect on the action of the two polypeptides oxytocin and vasopressin. In the case of angiotensin pregnancy has been shown to have some influence on its vascular action. Whether this can be attributed to an effect of the ovarian hormones does not appear to have been definitely looked into and this suggested itself as the initial line of experimentation. In view of the known effects of the ovarian hormones on water and electrolyte metabolism it was felt that an inquiry had also to be made into any effects of changes in the electrolyte composition of the extracellular fluid on the action of the polypeptide hormones.

The evidence from the use of blocking drugs as well as acute and chronic sympathectomy have shown that the withdrawal of sympathetic influences has a similar action to the presence of the ovarian hormones on the vascular effects of oxytocin and vasopressin. In the case of angiotensin sympathectomy has been shown to decrease the vasoconstrictor action in the hind limb of the dog and a great deal of work on different species indicate that it may interact with the sympathetic nervous system at a central, ganglionic and peripheral site. Yet the effect of blocking drugs on the vasoconstrictor action of angiotensin does not appear to show a consistent pattern (Nickerson, Bullock and Nomaguchi 1948; Supek et al 1962; Prado and Carlini 1959; Bianchi et al 1960; Yonkman, Jeremias and Stilwell 1943; Braun-Menendez and Fasiolo 1940;). Part of these differences are no doubt due to differences in the experimental set up and the species of animal used. It was therefore decided to determine the effect of blocking drugs on the pressor response to angiotensin in the rat and dog, the two

species used chiefly in this study and in the earlier work with oxytocin and vasopressin.

A peripheral interaction between angiotensin and the sympathetics have been shown by others. It appeared necessary to attempt to ascertain the exact site of this interaction. An isolated artery preparation was used with this end in view.

For convenience of presentation the results are divided into three sections, those obtained using rats, those obtained using dogs and those obtained using isolated arteries.

CHAPTER 4

EXPERIMENTS USING RATS

METHODS

Albino rats of approximately 200g body weight were used throughout to obviate the necessity for estimating comparable doses of drugs in different animals. Pentobarbitone 5mg/100g body weight was used as the anaesthetic in all animals. To avoid any obstruction to respiration the trachea was cannulated. A Condon mercury manometer writing on a smoked paper was used to register the blood pressure from a polyethylene cannula inserted into a common carotid artery. This cannula contained a small quantity of heparin solution to prevent clotting. A fine polyethylene cannula was also inserted into a femoral vein for the administration of drugs. Drugs were dissolved in isotonic saline so that the required dose was contained in 0.05 ml. This was then immediately washed in with 0.05 ml. of saline the dead space of the venous cannula being of the order of 0.02 ml. The angiotensin used was the synthetic valine 5 angiotensinamide (Ciba). When experiments involved the infusion of substances both femoral veins were cannulated and the infusions were made via one of the veins using a Palmer continuous infusion pump while the other vein was used for the administration of angiotensin. In experiments involving the collection of blood for electrolyte analysis, the animal was bled from an arterial cannula. Pseudopregnancy was induced by placing a vasectomised male in the cage with the females. The stage of the reproductive cycle and the onset of pseudopregnancy were ascertained by examining vaginal smears.

RESULTS AND DISCUSSION

1. Effect of ovarian hormones on the response to angiotensin.

The maximum elevations in the blood pressure produced by three different doses of angiotensin (2,4 and 8 nanograms), administered in random order, to female animals in different phases of the oestrus cycle, male, pseudo-pregnant and pregnant animals were measured. Preparations which did not give steady baseline blood pressures, making measurement of changes difficult, were discarded. This difficulty was encountered particularly in male animals. In the doses used there was no evidence of tachyphylaxis and the response in a given animal was reproducible. At least 5 mins. were allowed after the blood pressure returned to the basal value before administering another dose of angiotensin. The pressor response to a given dose did not appear to be related to the level of the basal blood pressure. An analysis of variance at each dose level showed that the mean responses of female animals in oestrus, dioestrus and pro-oestrus as well as of male animals did not differ significantly ($P>0.05$); although oestrous animals gave higher responses at all dose levels. The results of these groups were therefore pooled and used for comparison with other groups. The significance of difference was tested by an analysis of variance. The results are summerised in table 1 and figure 2.

Significantly lower responses than normal were observed in pregnant as well as pseudopregnant animals, particularly at the higher dose levels. (P values in table). Since a feature common to both pregnancy and pseudo-pregnancy is the presence of a functional corpus luteum, the possibility that the observed difference in the responsiveness was due to the presence of progesterone was considered next. Female rats were given progesterone in Arachis oil as a single subcutaneous injection in three different doses and tested with angiotensin 24 hours later. 0.2 and 0.6 mg. of progesterone

produced a reduction in the response to angiotensin but this reduction reached significance only at the lower dose. The addition of sodium oestradiol sulphate at two different doses did not produce any further change. This suggested that progesterone may be the basis for the reduced responses to angiotensin in pregnancy and pseudopregnancy. The possible mechanism of action of progesterone must be considered next.

Pregnancy is known to produce an increase in the extra-uterine weight (Newton 1935), and the humoral basis for this has been shown to be the secretion of progesterone (Dewar 1957a; Newton 1935; Hervey and Hervey 1967). This gain in weight is due to retention of water as well as an increase in the protein and fat content of the body. (Brooksby and Newton 1938; Dewar 1953; Dewar 1964; Hervey and Hervey 1967), and amounts to about 20% of the initial body weight. Hervey and Hervey (1967) have established that in their rats treated with progesterone the proportion of the increase in the weight attributable to water was 51% in one group and 61% in another. However, of this, only 22% in one instance and 28% in the other was retained without protein and ash, on the basis of the usual ratio of protein to water in the lean tissues. It is therefore only this amount of water that is likely to have been added to the extracellular fluid. This would lead one to expect an increase of approximately 5% in the blood volume. Bond (1948) has shown that after 21 days of gestation the blood volume of rats increased by 6.6%. Similar considerations apply in the case of pseudopregnancy (Dewar 1957b). Both the increase in the blood volume and the increase in the weight of the animal could have played a part in reducing the response to angiotensin since these animals received the same dose as the controls which were some 15 to 20g. lighter. It has however been noticed that the weight of the animal can vary between 150 to 300g. without any noticeable

change in the response to a given dose of angiotensin. Further the mean reduction in the pressor response (42.8% for pregnant and 30.4% for pseudopregnant) is much greater than the changes in the blood volume that can be expected. The observed differences are therefore unlikely to have arisen from this cause.

Hervey and Hervey (1967) have shown that the rate of increase in the weight as well as the total weight increase produced in rats by progesterone treatment is linearly related to the logarithm of the dose of progesterone for doses ranging from 0.2 to 10mg./day. If therefore the reduction in the responsiveness to angiotensin in the progesterone treated animals is also to be accounted for by a retention of water and the resultant increase in the blood volume, the higher doses of progesterone would be expected to produce a greater effect. This, however is not the case as the largest effect is seen at the intermediate dose of 0.2mg. Further, since these doses of progesterone were given only once and the animal tested 24 hours latter significant amounts of water are unlikely to have been retained. The doses of progesterone used are within the physiological range. Mayer (1963) and Miayake (1962) have found that 1 to 10mg./day of exogenous progesterone is required to secure implantation and continuance of pregnancy in rats that are ovariectomised soon after mating; 5mg/day of progesterone were required to reproduce a physiological situation similar to pregnancy in Hervey and Hervey's study, and at this dose level the plasma progesterone was about 0.06 to 0.12 µg/ml (Bullock and Cook 1967).

Another possibility is that progesterone in some way interferes with sympathetic transmission. In view of the numerous reports showing that in the absence of sympathetic nervous activity the response to angiotensin is reduced, this mechanism can explain the observed results. That

progesterone may act in this way has been suggested by the similarity in the effect of sympathectomy and progesterone rich states on the responses to oxytocin (Fullerton and Morrison 1965). There is also some evidence for an action of progesterone at the hypothalamic level (Barraclough 1962; Szentagothai et all 1962 Lloyd 1963) though not on the sympathetic outflow as such.

Kaplan and Silah (1964) and Gross Brunner and Ziegler (1965) have marshalled evidence to show that the sensitivity of the rat as well as other species to the vasoconstrictor effect of angiotensin varies inversely with the level of endogenous angiotensin and renin in the plasma. That progesterone might activate the renin angiotensin system is shown by the following considerations. Although some observations show that progesterone has a weak salt and water retaining action (Thorn and Engel 1938; Thorn Nelson and Thorn 1938; Gaunt, Nelson and Loomis 1938; Gaunt and Hays 1938), Landau and Lugibihl (1958) have shown that progesterone can act as an aldosterone antagonist at the renal tubular level thus giving rise to sodium depletion. Sodium depletion has been shown to be one of the factors quite consistently associated with an increased plasma renin level suggesting its increased secretion (Brown et all 1966). Direct evidence for an elevation of renin in pregnancy has been found by Fasciolo et all (1964) and Brown et all (1963), while Pickens et all (1965) have shown renin substrate level is also elevated in pregnancy. Laidlaw, Ruse and Gornall (1962) gave progesterone to six nonpregnant subjects and found that it produced increased urinary excretion of aldosterone in all of them and increased aldosterone secretion in two subjects in whom this was measured. Since the renin angiotensin system has been established as a stimulator of aldosterone secretion in man, it is reasonable to assume that renin secretion was also elevated in these

subjects. Similar results have been reported by Watanabe et al (1965). Winer (1965) reported that the daily administration of progesterone to dogs lead to a doubling of the plasma renin levels within four days.

The exact manner in which an increase in the endogenous renin production brings about a reduction in the sensitivity to exogenous angiotensin is not clear and several possibilities present themselves. Bock and Gross (1961) have demonstrated that cross tachyphylaxis occurs between renin and angiotensin when these substances are injected into an animal and this may account for the phenomenon under discussion. The effect of aldosterone, which may be produced by the increased levels of endogenous renin and angiotensin, on the vascular responsiveness to exogenous angiotensin is controversial. Katz et al (1963) have shown that in the rabbit and rat aldosterone administration markedly decreases the response to angiotensin. Ostrovsky and Gornall(1964) have reported a reduction in the duration of the pressor response rather than a reduction in the peak response after prolonged aldosterone treatment. On the other hand Kaplan and Silah (1963) have reported increased responsiveness to Angiotensin in primary hyperaldosteronism and McCaa et al(1967) have also reported an increased responsiveness in aldosterone treated dogs. Although the role of angiotensin in the secretion of aldosterone is well established in the case of the dog, there is some doubt whether it plays a similar role in other species, particularly the rat. Indirect data suggest an aldosterone stimulating action in the rat too but experiments designed to establish this have not succeeded (Cade and Perenich 1964; Marieb and Patrick 1965; Eilers and Peterson 1964). Therefore the vascular effect of a secondary elevation of aldosterone may not be relevant to the present discussion as these experiments were done on rats. The most likely explanation seems to be that the increase in the renin levels and the reduction in the responsiveness to angiotensin are effects arising from some other

common cause such as the reduction in the extracellular sodium. The experiments described under section 3 below were made in an effort to explore this aspect.

It may therefore be concluded that oestrogens do not influence the responsiveness to angiotensin while progesterone affects the vascular responsiveness to angiotensin in a direction opposite to that of vasopressin and oxytocin.

2. The effect of drugs blocking the action of the autonomic nervous system.

The mean maximum response to a dose of angiotensin was determined before and after the administration of the drug so that each animal acted as its own control except in the case of reserpine. The significance of the differences in the means was assessed by the 't' test. In the case of reserpine which was administered for two days (0.5mg/day), the mean responses were compared with those of the normal animals by analysis of variance. Dihydroergotamine (D.H.E.) and reserpine produced a lowering of the basal blood pressure while eserine produced a temporary elevation. The reserpine treated animals required only about 2/3 the usual dose of pentobarbitone.

The responses were significantly different only in the reserpine treated groups (see table for P Values). That this was not simply the result of a lower basal blood pressure was shown by the observation that reduction in the pressure caused by D.H.E., isoprenaline infusion or pithing did not have a similar effect. Because of the large number of possible sites of action of reserpine the interpretation of these results is hazardous. One possibility that must be considered is that in reserpine treated animals the cardiovascular buffer reflexes may be in abeyance so that greater pressor responses can occur. However if this were the case an effect in the same direction should occur in the case of D.H.E. and hexamethonium. To get more information on this effect of reserpine a pharmacological approach was made by studying

the effect of a variety of other drugs having related effects. The number of animals in these groups were too small for statistical treatment of the results which are summarised separately in table 3. Like reserpine, desmethylimipramine, guanethadine, bretylium and amphetamine all increased the response to angiotensin, while phenoxybenzamine did not show any great effect. Except for phenoxybenzamine all these drugs also produce an increased response to injected noradrenaline, desmethylimipramine, amphetamine bretylium and guanethadine probably by interfering with the re-uptake of noradrenaline into the nerve terminals (Axelrod, Whitby and Hertting 1961; Dengler, Spiegel and Titus 1961; Titus and Spiegel 1962;) and reserpine by a different mechanism (Trendelenburg 1963). Phenoxybenzamine has also been shown to interfere with the nerve membrane uptake of noradrenaline, but at the same time blocks the alpha receptor site so that the effect is not manifested as an increased sensitivity to injected noradrenaline. These findings are therefore consistent with the suggestion that at least part of the pressor action of angiotensin is due to a liberation of noradrenaline from a peripheral site. However, once again because of the multiple sites of action of these drugs interpretation is difficult. An attempt was made to define the effects of these drugs on the isolated hind limb vessels of the rat perfused with the rats own blood, but it had to be abandoned due to difficulties in getting steady basal perfusion pressures and consistent responses to angiotensin. Following reserpine treatment the time course of development of increased pressor sensitivity to angiotensin was followed in a series of animals. The earliest development of an increased sensitivity was observed about 12 hours after treatment and the effect was nearly complete 24 hours after treatment. Adrenalectomy did not influence the effect of reserpine.

In the effect following the administration of drugs blocking the sympathetic nervous system, therefore, angiotensin shows some similarity to vasopressin and oxytocin by producing enhanced pressor responses; but the mechanism underlying this increase in response is probably different in the case of angiotensin.

3. The effect of changes in extracellular ions.

The effect of acute changes in the sodium, potassium and calcium concentration in the extracellular fluid was studied. After preparation of the animal 0.5ml. of blood was collected by bleeding slowly via the arterial cannula which was then connected to the mercury manometer for recording the blood pressure. Several responses to 4nanograms of angiotensin were recorded and then either 2M disodium hydrogen sulphate solution (pH 7.4) or potassium chloride (40mg/ml) or calcium gluconate (10%w/v) solution was infused slowly over 20 to 30 minutes, and several pressor responses to the same dose of angiotensin recorded again. To produce a reduction in the extracellular sodium concentration Tyrode solution with its sodium chloride replaced iso-osmotically by sucrose was injected intraperitoneally and re-aspirated 25 to 30 minutes later, this being repeated two or three times. After this, the arterial cannula was replaced by another clean dry one and a second sample of blood collected through this. The electolyte concentrations of the serum was determined using a Unicam spectrophotometer. The significance of the differences between the means of the maximum pressor responses to angiotensin was assessed by the 't' test. The results are summarised in table 4. Significant differences were observed following an increase in potassium ($P=0.05$) and calcium ($P<0.01$).

These results show that either an increase or a decrease in the

extracellular sodium within the range of change achieved failed to influence the response to angiotensin to a significant degree ($P>0.05$). However, the responses after a decrease in the sodium do show a tendency if at all to be greater than the control period unlike when the sodium was elevated. This is in keeping with the common observation that the constrictor response of smooth muscle is potentiated by a decrease in the extracellular sodium (Napodano et al 1962; Friedman, Jamieson and Friedman 1959; Bohr, Brodie and Cheu 1958; Dodd and Daniel 1960). This would rule out the suggestion made in section 1 that progesterone brings about its effect of decreasing the pressor response to angiotensin by a sodium depleting action. Caution is necessary in interpreting the results in this way however since Hinke and Wilson (1962) have reported that in the rat tail artery preparation decreasing the extracellular sodium leads to a decrease in the response to angiotensin.

The finding that an increase in the extracellular potassium ion concentration results in an increase in the response to angiotensin is in agreement with the findings of others (Hinke and Wilson 1962). This may be interpreted as showing the importance of changes in membrane excitability in the action of angiotensin. Bohr, Brodie and Cheu (1958) have shown that a decrease of the K^+inside/K^+outside ratio by increasing the external potassium concentration increases the responsiveness of arterial smooth muscle to another vasoactive substance - adrenaline. The importance of the membrane potential has also received some confirmation by the work of Dodd and Daniel (1960). Several workers have shown that all classes of drugs that activate or inactivate smooth muscle cells have similar actions on their depolarised counterparts (Singh and Acharya 1957; Evans, Schild and Thesleff 1958). This however does not mean that the electrical activity at the membrane does

not contribute to the mechanical changes but rather that depolarised smooth muscle serves as a model preparation in which one component of the action of drugs can be studied in isolation. Thus Bohr (1964) has presented evidence to show that the drug induced tension development in vascular smooth muscle has a fast component, rate-limited by events occuring at the membrane and a slow component, rate-limited by the excitation contraction coupling process. He has proposed that the rate-limiting factor in the action of angiotensin is associated with membrane excitability.

Another interpretation of the enhancing effect of an increased extracellular potassium ion concentration on the response to angiotensin is suggested by the work of Read (1955). He has observed that angiotensin causes potassium to move into cells when the extracellular potassium concentration was high. There is also evidence that the entry of potassium into vascular smooth muscle cells is easier than into other cells and that an increase in the intracellular concentration of potassium leads to an increase in tension development (Laszt 1960). This is, however, contrary to the view proposed by Friedman and Friedman (1964) that the movement of sodium ions into the cell initiates contraction.

Calcium ions have been shown to play an essential role in the drug induced contraction of arterial smooth muscle by Waugh (1962b) and Bohr and Goulet (1961). Calcium has been associated with the chemico-mechanical transducing process in the contractile proteins of skeletal and cardiac muscle, and by analogy probably plays a similar role in vascular smooth muscle cells. A great deal of evidence points to the fact that the final common pathway by which drugs induce contraction of smooth muscle is by elevating the level of free intra-cellular calcium (Daniel 1963). This may be achieved either by increasing the permeability of the cell membrane so that extracellular calcium can reach the cell interior down a concentration gradient, or by the

liberation of bound calcium into the cell interior. The first possibility is considered to be more likely because in smooth muscle there is little endoplasmic reticulum and none of the complicated apparatus present in skeletal muscle which ensures the liberation of calcium close to the filaments of contractile protein. Further the diameter of the smooth muscle cell is so much less and the speed of response so much slower that simple diffusion of calcium may be a sufficient mechanism. This is probably the interpretation of the increased response to angiotensin in the presence of an elevated extracellular calcium found in this study.

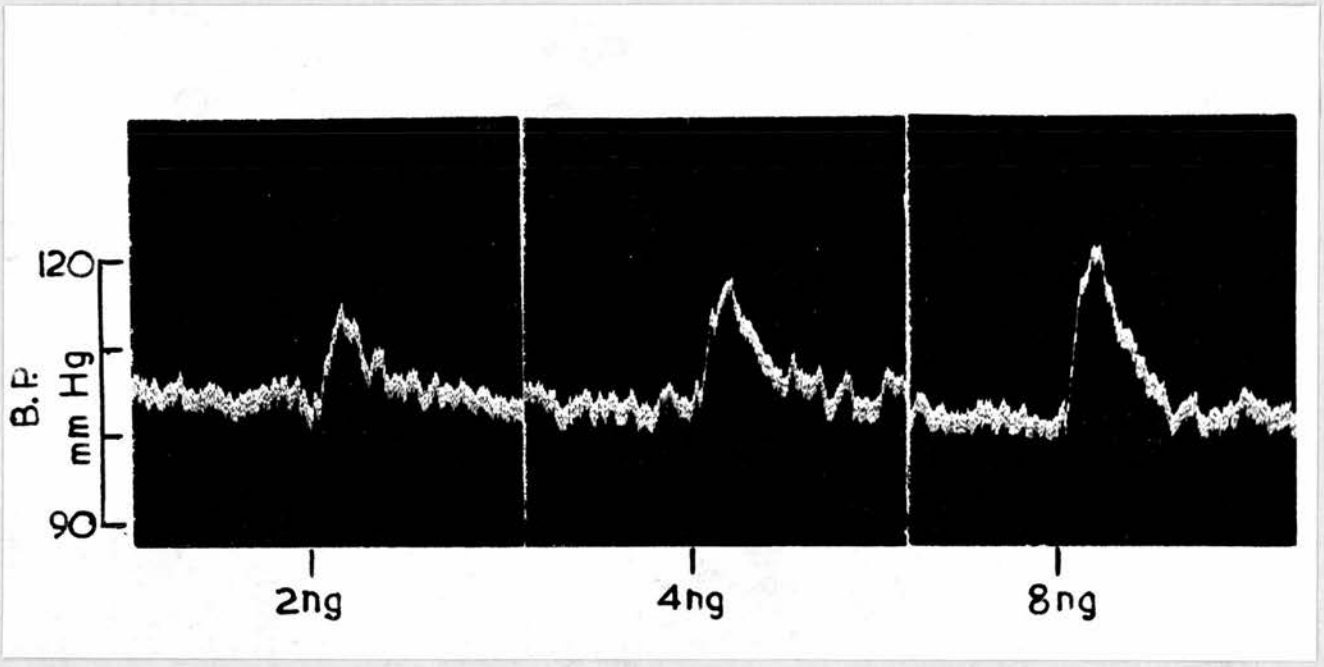


Figure 1.

Typical pressor responses to angiotensin in the rat.

TABLE 1

TYPE OF ANIMAL	ANGIOTENSIN ng	MEAN ELEVATION IN B.P.MM.Hg	No. OF ANIMALS	MEAN	STD. DEV. OF MEAN	STD. ERROR OF MEAN	P
OESTROUS	2	10.0; 5.0; 8.5; 9.5; 11.6; 10.7; 6.3; 5.7; 10.8;15.7; 14.3;	11	9.8	3.2	1.0	
	4	18.0;11.3; 9.4; 15.5;18.5; 22.3	6	15.8	4.4	2.0	
	8	23.5;13.5;13.2; 21.5;25.5;	6	21.3	6.2	2.5	
DI-OESTROUS	2	8.5;10.3;13.9; 7.3; 7.1; 8.1;12.0; 8.9; 10.3; 5.8; 6.5; 8.7;11.0;	13	8.3	2.4	0.7	
	4	9.0;10.8;18.0; 12.3;16.7; 9.3;12.5;13.3; 17.0;	9	13.2	3.2	1.1	
	8	15.5;25.0;17.0; 18.0;16.8; 14.8;19.0;20.5;	8	18.3	3.0	1.1	
PRO-OESTROUS	2	9.0; 6.1;14.4; 9.3;14.4; 8.4; 8.8; 8.3; 12;5; 9.2;	10	10.1	2.6	0.9	
	4	13.0;14.5;12.0; 19.5;15.3;	5	14.5	2.6	1.3	
	8	20.7;22.3;16.5; 25.7;19.0;	5	20.8	3.1	1.6	
MALES	2	8.9; 8.1; 8.9; 8.8;	4	8.7	0.3	0.2	
POOLED	2	-	38	9.5	2.5	0.4	
	4	-	20	14.3	3.6	0.8	
	8	-	19	19.9	4.5	1.1	
PREGNANT 12 DAYS	2	5.0; 6.1; 4.1; 4.1; 6.1; 8.0; 6.0;	7	5.6	1.3	0.5	<0.05
	4	8.6; 8.3; 4.0; 5.8; 7.9;11.4; 10.3;	7	8.0	2.3	1.0	<0.001
	8	11.1;11.6; 9.6; 9.0;12.1; 13.3;12.9;	7	11.3	1.5	0.6	<0.001

TABLE 1 Contd.

TYPE OF ANIMAL	ANGIOTENSIN IN ng.	MEAN ELEVATION IN B.P.MM.Hg	No. OF ANIMALS	MEAN	STD. DEV. OF MEAN	STD. ERROR OF MEAN	P
PSEUDO-PREGNANT 9-12 DAYS	2	7.8; 6.3; 6.2; 7.8; 8.3; 6.6; 8.7; 6.6; 6.0;	9	7.1	1.1	0.2	<0.05
	4	8.8; 8.4; 7.8; 10.6; 11.0; 10.5; 10.0; 8.1; 9.7;	9	9.4	1.7	0.3	<0.001
	8	12.7; 11.4; 10.7; 15.8; 14.8; 13.8; 17.0; 15.0; 13.0;	9	13.8	2.2	0.6	<0.001
PROGESTERONE 100 µg.	2	16.8; 11.3; 9.0; 9.6; 10.9; 11.7;	6	11.6	2.5	1.1	>0.05
	4	23.0; 16.0; 13.2; 15.2; 14.7; 15.6;	6	16.3	3.1	1.4	>0.05
	8	32.3; 19.2; 17.7; 25.7; 18.3; 26.8;	6	23.3	5.4	2.4	>0.05
PROGESTERONE 200 µg.	2	6.2; 8.3; 5.0; 8.2; 7.0;7.0;	6	7.0	1.1	0.5	>0.05
	4	9.7; 11.0; 10.7; 11.0; 12.0; 9.8;	6	10.7	0.8	0.4	<0.05
	8	12.7; 16.0; 14.2; 15.0; 13.0; 14.7;	6	14.3	1.4	0.6	<0.01
PROGESTERONE 600 µg.	2	8.0; 6.2; 4.9; 7.0; 8.7; 6.8;	6	6.9	1.2	0.5	>0.05
	4	11.7; 10.2; 7.7; 12.3; 15.7; 12.0;	6	11.6	2.4	1.1	>0.05
	8	17.0; 13.8; 12.5; 17.7; 21.3; 14.7;	6	16.2	2.9	1.3	>0.05
PROGESTERONE 200 µg + OESTRADIOL 150 µg.	2	5.9; 7.8; 6.0; 7.3; 6.8; 6.5;	6	6.7	0.7	0.3	>0.05
	4	9.3; 9.8; 10.7; 11.7; 12.2; 12.0;	6	10.9	1.1	0.5	<0.05
		13.3; 13.0; 15.5; 16.5; 15.0 16.2	6	14.9	1.3	0.6	<0.05

TABLE 1 Contd.

TYPE OF ANIMAL	ANGIOTENSIN IN ng.	MEAN ELEVATION IN B.P.MM.Hg	No. OF ANIMALS	MEAN	STD. DEV. OF MEAN	STD. ERROR OF MEAN	P
PROGEST-ERONE	2	7.5; 9.0; 6.2; 7.2; 8.0; 6.0;	6	7.3	1.0	0.4	>0.05
600 µg. OESTRA DIOL	4	12.8; 11.9; 9.5; 12.0; 14.8; 11.0;	6	12.0	1.6	0.7	>0.05
400 µg.	8	16.0; 15.0; 13.3; 17.3; 19.2; 14.0;	6	15.8	1.5	0.7	>0.05
RESER-PINE	2	12.7; 13.7; 15.0; 19.5; 13.1;	5	14.8	2.5	1.2	<0.01
0.5 mg. DAILY	4	17.3; 17.3; 22.0; 26.2; 21.0	5	20.8	3.5	1.8	<0.01
FOR 2 DAYS	8	26.5; 22.5; 32.8; 37.0; 31.3;	5	30.0	5.0	2.5	<0.001

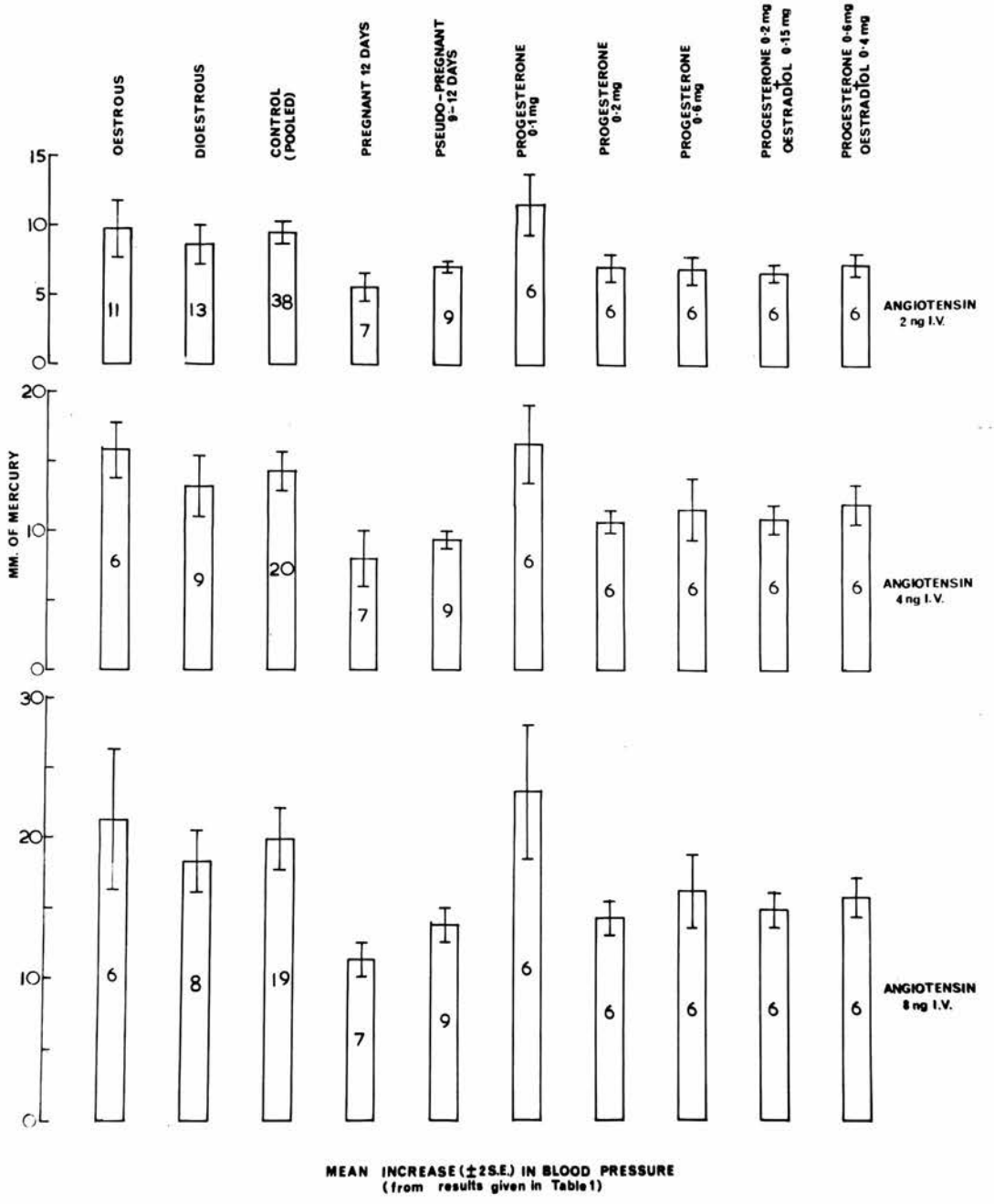


Figure 2.

Effect of the oestrous cycle, pregnancy, pseudopregnancy and progesterone administration on the presser action of angiotensin in rats (Data given in table 1).

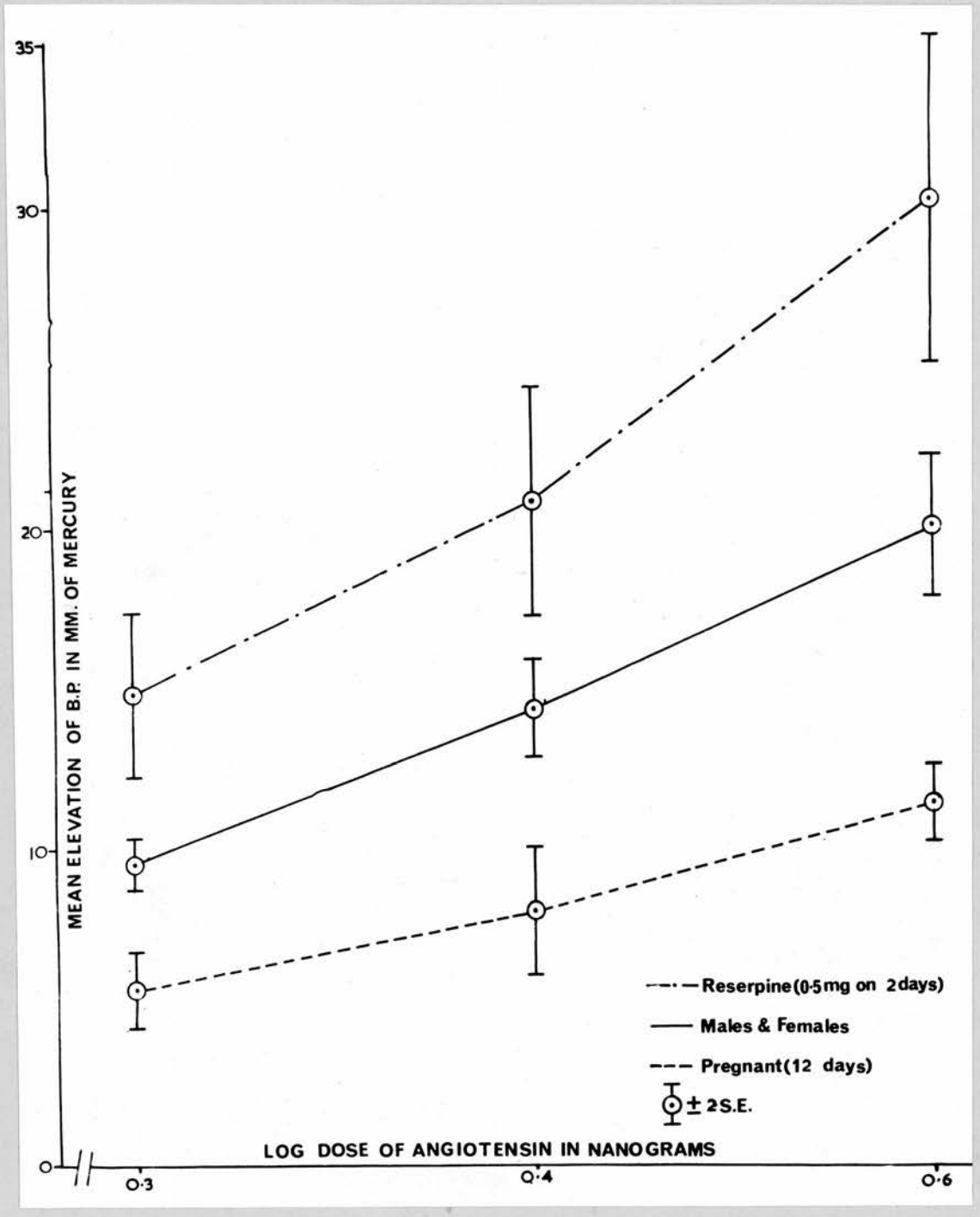


Figure 3.

Dose-response relationship of pressor action of angiotensin in normal, pregnant and reserpine treated rats. (Data in table 1 and 2).

Table 2

DRUG GIVEN	ANGIOTENSIN ng.		MEAN MAXIMUM ELEVATION OF. B.P. IN MM. Hg.	No. of ANIMALS	MEAN	STD. DEV. OF MEAN	STD. ERROR OF MEAN	P
DIHYD-ROERG-OTAMI-NE 0.1mg I.V.	2	B	13.9; 10.3; 5.7; 5.7; 10.8; 9.2;	6	9.3	2.9	1.3	>0.05
		A	11.0; 7.3; 6.8; 6.4; 10.5; 10.0;		8.7	1.8	0.8	
	4	B	16.7; 9.4; 9.3; 15.5; 19.5	5	14.1	4.0	2.0	>0.05
		A	15.0; 11.8; 10.4; 14.8; 12.0;		12.8	1.8	0.9	
	8	B	18.9 13.3 16.8; 21.5; 25.7	5	19.1	4.2	2.1	>0.05
		A	23.0; 17.2; 16.6; 20.7; 19.9;		19.3	2.3	1.1	
PRO-NETH-ANOL 1 - 2 mg. I.V.	2	B	8.7 8.5; 14.0; 11.0; 13.8; 14.3;	6	11.7	2.4	1.1	>0.05
		A	5.8; 11.0; 16.0; 9.9; 7.0; 10.5;		10.0	3.3	1.5	
	4	B	12.6; 12.0; 18.5; 17.0; 13.3; 22.5;	6	15.9	3.7	1.6	>0.05
		A	14.5; 14.8; 26.0; 17.6; 12.0; 19.8;		17.5;	4.4	2.0	
	8	B	19.0; 16.5; 25.5; 20.5; 19.0; 30.3;	6	21.8	4.7	2.1	>0.05

B = Before administration of drug

A = After administration of drug

TABLE 2 CONTD.

DRUG GIVEN	ANGIOTENSIN ng.		MEAN MAXIMUM ELEVATION OF B.P. IN MM. Hg.	No. OF ANIMALS	MEAN	STD. DEV. OF MEAN	STD. ERROR OF MEAN	P
ATROPINE 1-2 mg. I.V.	2	B	8.9; 8.9; 8.4; 8.8; 6.3;	5	8.3	1.0	0.5	>0.05
		A	8.5; 6.8; 8.3; 8.8; 4.3;		7.3	1.8	0.9	
	4	B	12.5; 13.1; 14.5; 11.3;	4	12.9	1.1	0.7	>0.05
		A	9.0; 17.6; 13.5; 7.4;		11.9	4.0	2.3	
	8	B	17.0; 22.7; 22.3; 13.5;	4	18.4	3.4	2.0	>0.05
		A	13.0; 22.8; 18.5; 11.1;		18.9	5.3	3.0	
HEXAMETHONIUM 0.5-1 mg.	2	B	9.5; 10.3; 8.8; 14.5; 12.0;	5	11.0	2.0	1.0	>0.05
		A	10.9; 8.2; 6.9; 9.0; 13.8;		9.8	2.3	1.1	
	4	B	18.0; 18.0;	2	18.0	0	-	>0.05
		A	25.5; 12.8;		18.9	6.1	-	
	8	B	25.0; 23.5;	2	24.3	0.8	-	>0.05
		A	39.0; 16.5;		27.8	11.3	-	
ESERINE 30-60 µg. I.V	2	B	8.5; 13.9; 5.8;	3	9.4	3.3	2.4	>0.05
		A	6.5; 9.8; 5.8;		7.4	1.7	1.2	

B = Before administration of drug.

A = After administration of drug.

TABLE 3

DRUG	TIME AFTER ADMINISTRATION IN MINUTES.	DOSE OF ANGIOTENSIN	MEAN MAX. RESPONSE MM. Hg. BEFORE.	AFTER
DESMETHYLIMIPRAMINE 1-2 mg.	40 mins.	2ng 4ng	10.4 13.5	17.5 22.1
AMPHETAMINE ½ mg.	30 mins.	2ng	17.6	39.3
GAUNETIDINE 4-6 mg.	120 mins.	2ng 4ng 8ng	6.3 10.0 14.5	15.5 27.3 44.0
BRETYLIUM 2-3 mg.	60 mins.	2ng 4ng	10.3 19.0	24.4 35.4
PHENOXYBENZAMINE 1-2 mg.	30 mins.	2ng	7.8	9.1
ALPHAMETHYLDOPA 10 mg.	90 mins.	2ng 4ng	11.3 13.9	18.8 17.3

TABLE 4

MEAN RESPONSE TO 4ng ANGIOTENSIN MM. Hg		CHANGE IN BASAL B.P.MM.Hg.	SERIUM ION CONC. meq/l		ION ADDED mg	VOL. INF. ml	P
BEFORE	AFTER		BEFORE	AFTER			
INCREASED SODIUM							
14.0	12.0	-65	152.2	160.0	32.1	0.7	
8.7	8.3	-48	134.8	142.6	36.7	0.8	> 0.05
10.7	10.0	-60	120.9	130.4	32.1	0.7	
7.0	7.3	-35	138.3	145.7	32.1	0.7	
DECREASED SODIUM							
16.3	19.3	-30	101.3	90.4			
17.1	15.4	=	110.4	106.5			> 0.05
13.0	23.5	-60	110.4	88.7			
16.0	16.1	+14	113.0	93.5			
INCREASED POTASSIUM							
4.3	15.8	-64	5.8	8.3	4.2	0.2	
7.0	22.0	-42	4.3	5.7	3.2	0.15	
10.8	19.8	-38	4.4	5.2	4.2	0.2	= 0.05
14.0	8.0	-14	4.9	5.2	2.1	0.1	
9.3	21.8	-36	3.9	5.8	2.1	0.1	
11.3	13.1	-38	4.6	5.8	2.1	0.1	
INCREASED CALCIUM							
9.2	13.1	-30	5.3	6.7	5.3	0.6	
7.4	9.6	-48	5.3	5.8	3.5	0.4	
10.6	14.2	-45	5.3	7.2	3.5	0.4	< 0.01
10.7	14.0	-28	4.8	5.7	0.9	0.1	
9.3	14.5	-34	4.5	5.4	0.9	0.1	

= No change
+ Increased
- Decreased.

CHAPTER 5

EXPERIMENTS USING DOGS

METHODS.

(a) Effect of angiotensin on vascular resistance in the hind limb.

The dogs were anaesthetised with intravenous pentobarbitone (130mg/Kg); small doses being administered subsequently during the experiment to maintain the level of anaesthesia. The abdomen was opened through a mid line incision and the lumbar sympathetic chain on one side identified. The last lumbar ganglion was located and two loose ligatures placed distal to it for subsequent identification and tying. The femoral arteries were exposed in the femoral triangles and each one was cannulated proximally and distally. Each proximal cannula was connected to its corresponding distal cannula by plastic tubing which passed through a constant flow Sigmamotor pump. Two double limb mercury manometers connected to a point distal to the pump were used to record the perfusion pressures of the limbs. The systemic pressure was measured by a third manometer connected to one of the proximal cannulae in one of the femoral arteries. The flow in the arteries was occluded only briefly during the cannulation and connecting up procedures and the animal was heparinised (5mg/kg) before flow through the external circuit was started. The speed of the pump was adjusted initially to give a perfusion pressure in the limbs equal to the systemic pressure. The external tubing was water jacketed and each side had a volume of approximately 20 mls. Intra-arterial injections were made through short lengths of rubber tubing between the distal arterial cannula and the plastic tubing. Intravenous injections were made into a cannulated femoral vein. A dose of angiotensin which gave an elevation in the perfusion pressure of about 20mm. of mercury on intra –

arterial injection was selected and used throughout each experiment. Ten to fifteen minutes after the operative procedure was completed responses of both limbs to the intra arterial injection of the selected dose of angiotensin was recorded. The sympathetic trunk on one side was then tied, using the ligatures placed before hand, and sectioned between the ligatures. The responses to the same intra-arterial dose of angiotensin was recorded subsequent to this. Bipolar electrodes were placed on the distal end of the cut sympathetic nerve, isolated from surrounding tissues and stimulated using pulses of 1 msec duration. Responses to angiotensin were recorded both during and after stimulation of the nerve. The effect of a variety of drugs on the response to angiotensin was then tested.

(b) The effect of generalised sympathetic activity on the pressor action of angiotensin.

Anaesthesia was induced using ether, and intravenous chloralose used for maintainence, and the dog prepared as in (a) above. Attempts to increase the general sympathetic discharge by brief occlusion of the carotid arteries failed as shown by a lack of elevation in the systemic blood pressure. An elevation of the systemic pressure was however acheived by distention of the bladder with warm saline solution. Responses to intravenous and intra-arterial angiotensin were recorded both before and during such distention of the bladder.

(c) Effect of angiotensin on sodium ion movement.

Dogs under pentobarbitone anaesthesia were used. Through a mid line abdominal incision the renal pedicles were cleared and firm ligatures tied round them to occlude the renal arteries. Raffinose (60mg/kg.) was then administered intravenously. An internal jugular vein and a carotid artery were cannulated in the direction of the heart. Blood from the carotid

artery was lead by means of plastic tubing through a sigmamotor pump to either an iliac or femoral artery cannulated in the direction of the limb. The perfusion pressure of the limb and the systemic pressure were measured by double limb mercury manometers recording on smoked paper connected distal to and proximal to the pump respectively. The iliac or femoral vein of the perfused limb was cannulated and the blood leaving the limb was lead through a specially designed chamber containing a sodium electrode. From this chamber the blood was returned to the circulation via the cannulated internal jugular vein. A short side arm in the tubing beyond the chamber containing the sodium electrode enabled samples of blood to be collected when the electrode indicated that changes in the sodium concentration were occuring. The external tubing had a volume of about 50 ml. The tubing and the chamber containing the sodium electrode were water jacketed and maintained at a constant temperature of about 38°C. The animal was heperinised (5mg/kg) before flow through the external circuit was started. Angiotensin and other drugs were dissolved in a solution of four parts of isotonic saline to one part of 5% dxtrose solution. Preliminary trials had shown that this mixture did not affect the sodium electrode when injected into the circulation of the limb by itself. The samples of blood that were collected were centrifuged, the plasma pipetted off and analysed for sodium and raffinose concentrations. Sodium concentration was determined on a Unicam flame sprectrophotometer (SP.900) and the following procedure was adopted for analysis of raffinose (modification of method described by Higashi and Peters 1950). 6ml. of distilled water and 2ml. of trichlor acetic acid (13%) were added to 0.2ml. of the plasma and the mixture centrifuged after shaking up. To 1ml. of the supernatant was added 1ml. of resorcinol (0.1g/100ml. ethanol), the mixture cooled in ice and 3ml. of concentrated

HCl (S.G. 1.19 containing 7.5mg. $FeCl_3$/l) then added. The solution was then transferred to a water bath at 80°C for 10 minutes at the end of which the reaction was stopped by cooling in ice and the optical density of the solution measured in a Unicam spectrophotometer (SP.500) at a wavelength of 480mμ. The concentration of raffinose was derived from a comparison with solutions containing standard amounts of raffinose.

RESULTS AND DISCUSSION

(a) Effect of angiotensin on the vascular resistance of the kind limb.

Under the conditions of constant flow obtaining in these experiments the changes in the perfusion pressure of the limb are proportional to changes in the vascular resistance. The protocols of the experiments are given in table 5(a) to (e).

The effect of sympathectomy alone on the perfusion pressure of the limb was variable. In three experiments the perfusion pressure was reduced indicating a vasodilatation; In two of the experiments there was no change in the perfusion pressure after sectioning the sympathetic trunk. The effect of sympathectomy on the response to angiotensin was also variable. On two occasions it was reduced on two occasions there was no effect and in one instance there was a marked enhancement of the response. There was also no correlation between the change in resting perfusion pressures following sympathectomy and these effects on the response to angiotensin.

The effect of stimulation of the distal end of the severed sympathetic trunk was to elevate the perfusion pressure indicating a vasoconstriction in the limb vasculature on the side concerned. An interesting effect was noticed in experiment (e) where stimulation of the sympathetics lead, in addition, to a fall in the perfusion pressure in the opposite limb indicating a vasodilatation there (Figure 5). This was noticed twice at two different strengths of stimulation and was abolished when atropine was administered intravenously. Cholinergic vasodilator fibres have been shown to exist in the sympathetic outflow to the lower limb of the dog by several workers (Bulbring and Burn 1935; Furmin, Ngai and Wang 1953; Clonninger and Green 1955; Youmans, Green and Denison 1955) but these results would seem to suggest that there is a crossing over of these fibres at a low level as well.

The effect of nerve stimulation on the response to angiotensin was again variable. In general the direction of change was opposite to the direction of change following sympathetomy; where sympathectomy had reduced the response stimulation of the nerve tended to restore it, while where there had been no change, stimulation too had no effect. In experiment (e) where there was a vasodilatation in the opposite limb, the response to angiotensin in that limb was definitely potentiated during nerve stimulation and this effect was once again abolished by atropine administration.

These results suggest that the relationship between the sympathetic nerve supply and the response of the hind limb vessels of the dog to angiotensin is not as straight forward as suggested by the work of Zimmerman (1962). To the extent that stimulation of the nerve tends to counteract any effect that sympathectomy had on the response to angiotensin, the sympathetics may be said to influence the response to angiotensin, but in these results the direction of this influence seems to be quite variable. The enhancing effect of an activation of the cholinergic vasodilator fibres as seen in experiment (e) leads one to speculate whether this effect may account in some way for the differences in this study and that of Zimmerman.

If angiotensin is assigned the ability to either liberate noradrenaline from sympathetic nerve endings or to enhance its release from this site or to interfere with the re-uptake and inactivation process of noradrenaline one would have expected far more clear cut results from this experimental preparation. Experiment (e) shows also that even an infusion of angiotensin failed to influence the constriction due to sympathetic stimulation.

Calcium gluconate and desmethylimipramine did not influence the response to angiotensin. An infusion of noradrenaline was similarly ineffective.

An intra-arterial infusion of adrenaline increased the response to angiotensin on two occasions but on two others it had little or no effect. Thus unlike the case of oxytocin an adrenaline infusion after sympathectomy, in the presence or absence of atropine fails to have a consistent effect on the response to angiotensin.

Dihydroergotamine was found to increase the response to intra-arterial angiotensin more specially on the side that had been denervated (Fig. 4). The interpretation of this finding is doubtful. Bevis and Mohme-Lundholm (1966) have shown that in isolated bovine arteries dihydroergotamine at a concentration of 10^{-6} increases the concentration of ATP and CP. This was accompanied by a gradual increase in the tension of the artery. In this study too D.H.E. intra-arterially invariably produced an elevation of the vascular resistance and the increased responses to angiotensin in the isolated perfused limb may be related to the elevation in the energy rich organic phosphate compounds in the vascular smooth muscle cells.

(b) The effect of generalised sympathetic activity on the pressor action of angiotensin.

The results of the few experiments that were done were entirely negative. No difference in the response to angiotensin administered intravenously or intra-arterially into the limb circulation was observed either after or during a generalised increase in sympathetic activity. Again some difference would have been expected if angiotensin influenced the events occuring at the sympathetic nerve terminals.

(c) The effect of angiotensin on ion movement.

Friedman, Butt and Friedman (1957) have shown that the vasoconstrictor action of angiotensin is accompanied by a decrease in the extracellular

sodium ion content. A similar change was produced by other vasoconstrictors, while vasodilators had the opposite effect. They have proposed on the basis of subsequent work (Friedman and Friedman 1964) that these movements of sodium are causal in the production of constriction and relaxation, of the vascular smooth muscle cells. In these studies water was found to move with the sodium. The necessity for such a movement of water however can be obviated if the inward movement of sodium is accompanied by a simultaneous outward movement of a corresponding amount of potassium. It has been found by the same workers that vasoconstriction due to vasopressin and noradrenaline is accompanied by an outward movement of potassium but the constriction due to angiotensin did not produce any shifts in the potassium. It would therefore be expected that angiotensin should produce the largest shifts in water. However in the studies of Friedman and co-workers water movement accompanying the inward shift of sodium was minimal in the case of vasopressin so that with this drug the extracellular sodium concentration decreased most; with noradrenaline the shift in water closely paralled the shift in sodium so that plasma concentration of sodium hardly changed, while with angiotensin the change in the plasma sodium was intermediate to that caused by vasopressin and noradrenaline. It had been noticed earlier (Lloyd and Pickford unpublished observations) that in the hind limb circulation of the dog an intra-arterial injection of oxytocin produced an increase in the plasma sodium - interpreted as a movement of sodium out of the vascular smooth muscle cells - accompanying the vasodilatation. This was in agreement with the hypothesis proposed by Friedman et al.. When however the dilator action of oxytocin was converted to one of constriction by the administration of an alpha blocking agent the shifts in sodium accompanying the vasoconstriction were the same as that during the

previous vasodilatation on many occasions. For these two reasons and because of the reported effects of the sympathetics on the angiotensin response, it was decided to investigate ion and water shifts in the hind limb preparation of the dog during angiotensin induced vasoconstriction in the presence and absence of blocking agents. Further since the shifts in ions following noradrenaline administration have been reported to be abolished by alpha blocking agents and since these substances are believed to act competitively by attachment to the receptor site it was of interest to see whether the blocking agents themselves produce any ion shifts.

The method used to study these changes in the hind limb of the dog is basically similar to that used by Jamieson & Friedman (1961) except for the additional use of raffinose and blood sampling in order to estimate not only shifts in sodium but also shifts in water. The fixed quantity of angiotensin, selected for each experiment on the basis of an adequate constrictor response in the limb without influencing the systemic pressure, was injected through out into the tubing between the Sigmamotor pump and the arterial cannula. The sodium electrode did not always show the expected change in the sodium concentration even after allowance was made for the delay in flow between the venous side of the limb and the electrode. Blood samples were therefore collected from the venous side at a fixed interval of time after the angiotensin injection shown by trial to allow for the delay in the flow. The volume of the samples were the same through out an experiment.

The raffinose concentration of a sample was converted to a percentage change as compared with that of a control sample taken at the beginning of the experiment, that is after about one hour had been allowed for the raffinose to equilibriate. After blocking agents fresh controls were

taken and subsequent samples compared with these. An increase in the raffinose concentration was denoted by a + and would indicate a shift of water out of the extracellular compartment. Raffinose was used instead of the more conventional inulin as it was hoped its smaller molecular size would enable the vascular and interstitial compartments to come into equilibrium more rapidly so that the simplifying assumption that changes in the plasma concentration is equal to the extracellular fluid concentration could be made. The changes in sodium concentration were similarly expressed as a percentage of the control value, + denoting an increase and - denoting a decrease. These changes in concentration were however influenced by the shifts in the water, so that the true changes in the sodium concentration were calculated from the expression:

True per cent change in Na^+ conc. = Observed per cent change in Na^+ conc. - Per cent change in raffinose conc.

Too much cannot be read into these results however as there are possible errors in the simplifying assumptions and above all steady state conditions were not being observed and continuously varying exchanges are being quantified by the discontinuous process of sampling. However the results should be valid as far as the broad pattern of changes go. The results are summarised in table 6 and in figs 6 & 7.

These results show that angiotensin does not induce shifts in sodium or water consistently either into or out of the cells. The blocking agents by themselves do not appear to effect any consistent pattern of ion shifts nor do they appear to affect the ion shifts due to angiotensin. These results are therefore not in agreement with those of the Friedmans' and suggest that the movement of sodium ions are not directly related to the constrictor effect of angiotensin. This is in keeping with the data of Turker, Page and Khairallah (1967) who found that in the canine arterial

smooth muscle angiotensin did not influence the rate of influx of Na^{22} or its equilibrium level but increased the Na^{+} efflux correlated with the contraction. Other drugs were found by these workers to influence the Na fluxes in different ways and these could be modified by various means without affecting the mechanical actions induced by these drugs.

TABLE 5 a

DRUG ADMINISTERED OR OTHER PROCEDURE	CHANGE IN LEFT LEG PERFUSION PR.MM.Hg	CHANGE IN RIGHT LEG PERFUSION PR.MM.Hg.
0.5µg Angiotensin I.A.	+ 28	+ 35
"	*	+ 42
SYMPATHECTOMY	*	- 26
0.5µg Angiotensin I.A.	+ 23	+ 20
"	+ 19	+ 22
STIMULATION OF NERVE (8/sec 5 V)	*	+ 24
0.5µg Angiotensin I.A.	+ 20	+ 26
STIMULATION OFF	*	- 20
0.5µg Angiotensin	*	+ 10
STIMULATION ON	*	+ 21
0.5µg Angiotensin I.A.	*	+ 20
STIMULATION OFF	*	- 21
0.5µg Angiotensin	*	+ 20
STIMULATION ON (8/sec 1 V)	*	+ 4
0.5µg Angiotensin I.A.	+ 7	+ 16
STIMULATION OFF	*	- 4
1.0µg Angiotensin I.A.	+ 10	+ 10
"	+ 17	+ 13
"		+ 13
"	*	+ 13
Desmethylimipramine 1.5mg I.A.	*	- 52 & + 52
1.0µg Angiotensin	*	+ 11
"	*	+ 12
Desmethylimipramine 1.5mg I.A.	- 24 & + 24	*
1.0µg Angiotensin I.A.	+ 18	*
"	+ 12	*
"	+ 16	*
Dihydroergotamine 1.0mg I.V.	+ 16	+ 26
1.0mg Angiotensin I.A.	+ 38	+ 26
Pronethanol 2mg I.V.	=	=
1.0mg Angiotensin I.A.	*	+ 18
Dihydroergotamine 1.5mg I.V.	=	=
1.0mg Angiotensin I.A.	+ 28	+ 24
"	+ 25	+ 23
"	*	+ 21

* Not applicable
= No change
\+ Increase
\- Decrease

TABLE 5 b

DRUG ADMINISTERED OR OTHER PROCEDURE	LEFT LEG PERFUSION PR. MM. Hg	RIGHT LEG PERFUSION PR. MM.Hg.
0.5µg Angiotensin I.A.	+ 30	+ 18
SYMPATHECTOMY	*	=
0.5µg Angiotensin	+ 14	+ 12
STIMULATION OF NERVE (4/sec 1.5 V)	*	+ 10
0.5µg Angiotensin I.A.	+ 28	+ 18
STIMULATION OFF	*	- 10
Noradrenaline 0.1µg/mt I.A.	*	+ 6
0.5µg Angiotensin I.A.	*	+ 16
Noradrenaline infusion off	*	- 6
0.5µg Angiotensin I.A.	*	+ 17
Calcium gluconate 0.5mg/mt I.A. infusion	*	=
0.5µg Angiotensin	*	+ 14
STIMULATION ON	*	=
0.5µg Angiotensin		+ 20
CALCIUM & STIMULATION OFF	*	=
Angiotensin 0.1µg/mt I.A. Infusion	*	+ 6
STIMULATION ON FOR 1 min	*	+ 14
"		+ 18
Angiotensin infusion off		- 6
0.5µg Angiotensin I.A.	+ 36	*
STIMULATION ON FOR 1 min	*	+ 18
Noradrenaline 0.1µg/mt Infusion	+ 10	*
0.5µg Angiotensin I.A.	+ 36	*
Noradrenaline infusion off	- 10	*
Calcium gluconate 0.5mg/mt I.A. infusion	=	*
0.5µg Angiotensin I.A.	+ 34	*
Calcium infusion off	=	*
Adrenaline 2.5ug/mt I.A. Infusion	-20 & + 20	
0.5µg Angiotensin I.A.	+ 34	*
Adrenaline infusion off	=	*
Adrenaline 2.5µg/mt I.A. Infusion	*	+ 44
0.5µg Angiotensin I.A.	*	+ 49
Adrenaline infusion off	*	- 40
0.5µg Angiotensin I.A.	*	+ 18
Adrenaline 0.5ug/mt I.A. Infusion	*	+ 20
0.5µg Angiotensin	*	+ 37
Adrenaline infusion off	*	- 20
Dihydroergotamine 1mg I.V.	- 44 & + 44	+ 30
0.5µg Angiotensin I.A.	+ 44	+ 44
STIMULATION ON	*	- 20 & + 20
0.5µg Angiotensin I.A.	*	+ 50

* Not applicable
= No change
+ Increase
- Decrease

TABLE 5 c

DRUG ADMINISTERED OR OTHER PROCEDURE	LEFT LEG PERFUSION PR.MM.Hg.	RIGHT LEG PERFUSION PR. MM.Hg.
0.5µg Angiotensin I.A.	+ 10	+ 24
SYMPATHECTOMY	- 10	*
0.5µg Angiotensin I.A.	+ 10	+ 14
STIMULATION OF NERVE (6/sec 5 V)	+ 2	*
0.5µg Angiotensin I.A.	+ 10	+ 14
STIMULATION OFF	- 2	*
Adrenaline 0.25µg/mt I.A. infusion	+ 16	*
0.5µg Angiotensin I.A.	+ 16	+ 12
Adrenaline infusion off	- 16	*
Adrenaline 0.25µg/mt I.A. infusion	*	=
0.5µg Angiotensin	*	+ 8
Dihydroergotamine 1 mg I.V.	+ 13	+ 32
0.5µg Angiotensin I.A.	+ 13	+ 11
Dihydroergotamine 0.1mg I.A.	=	*
0.5µg Angiotensin I.A.	+ 14	+ 14
Adrenaline 0.25µg/mt I.A. infusion	- 4	*
0.5µg Angiotensin I.A.	+ 14	+ 12

* Not applicable
= No change
+ Increase
- Decrease

TABLE 5 d

DRUG ADMINISTERED OR OTHER PROCEDURE	LEFT LEG PERFUSION PR.MM.Hg.	RIGHT LEG PERFUSION PR.MM.Hg.
0.5µg Angiotensin I.A.	+ 17	+ 34
SYMPATHECTOMY	=	*
0.5µg Angiotensin I.A.	+ 20	+ 33
STIMULATION OF NERVE (8/sec 5 V)	+ 12	*
0.5µg Angiotensin I.A.	+ 16	+ 34
Adrenaline infusion 0.6µg/mt I.A. Infusion	+ 2	*
0.5µg Angiotensin I.A.	+ 20	+ 36
Nembutal I.V.	- 8	- 20
0.5µg Angiotensin I.A.	+ 18	+ 28
Adrenaline infusion off	=	*
Adrenaline 0.6µg/mt I.A. infusion	*	=
0.5µg Angiotensin I.A.	+ 17	+ 28
Adrenaline infusion off	*	=
Phentolamine 2.5mg I.V.	- 16	- 36
0.5µg Angiotensin	+ 20	+ 32
Dihydroergotamine 1 mg I.V.	+ 25	- 14 & + 14
0.5µg Angiotensin I.A.	+ 23	+ 32
STIMULATION OF NERVE	-16 & + 16	*
0.5µg Angiotensin I.A.	+ 26	+ 38
STIMULATION OFF	=	*
0.5µg Angiotensin I.A.	+ 36	+ 38

* Not applicable
= No change
+ Increase
- Decrease

TABLE 5 e

DRUG ADMINISTERED OR OTHER PROCEDURE	LEFT LEG PERFUSION PR.MM.Hg.	RIGHT LEG PERFUSION PR.MM. Hg.
0.25µg Angiotensin	+ 18	+ 22
SYMPATHECTOMY	- 38	*
0.25µg Angiotensin I.A.	+ 32	+ 20
STIMULATION OF THE NERVE (6/sec 2 V)	+ 36	- 24
0.25µg Angiotensin I.A.	+ 18	+ 30
STIMULATION OFF	- 48	+ 14
0.25µg Angiotensin I.A.	+ 24	+ 22
PERFUSION RATE INCREASED	+ 48	+ 38
0.25µg Angiotensin I.A.	+ 22	+ 18
PERFUSION RATE REDUCED TO FORMER VALUEA	- 48	- 38
0.25µg Angiotensin I.A.	+ 21	+ 12
STIMULATION ON (2/sec 0.25 V)	+ 22	- 12
0.25µg Angiotensin I.A.	+ 12	+ 22
STIMULATION OFF	- 32	+ 12
Atropine 4mg I.V.	=	=
0.25µg Angiotensin I.V.	+ 22	+ 16
STIMULATION ON (2/sec 0.25 V)	+ 8	=
0.25µg Angiotensin I.A.	+ 14	+ 10
STIMULATION OFF	- 12	=
Adrenaline 0.6µg/mt I.A.	=	=
0.25µg Angiotensin I.A.	+ 11	+ 10
Bretylium 100mg I.V.	- 70 & + 70	- 70 & + 70
0.25µg Angiotensin I.A.	+ 12	+ 16
"	+ 10	+ 16
STIMULATION (2/sec 0.25V)	+ 19	=
0.25µg Angiotensin I.A.	+ 12	+ 10
Dihydroergotamine 1mg I.V.	+ 22	+ 28
0.25µg Angiotensin I.A.	+ 22	+ 22
"	+ 28	+ 31
Noradrenaline 25 µg I.V.	+ 16	+ 22
Dihydroergotamine 3mg I.V.	- 20	- 28
0.25µg Angiotensin	+ 26	+ 28
Noradrenaline 25µg I.V.	+ 6	+ 4

* Not applicable
= No change
+ Increase
- Decrease

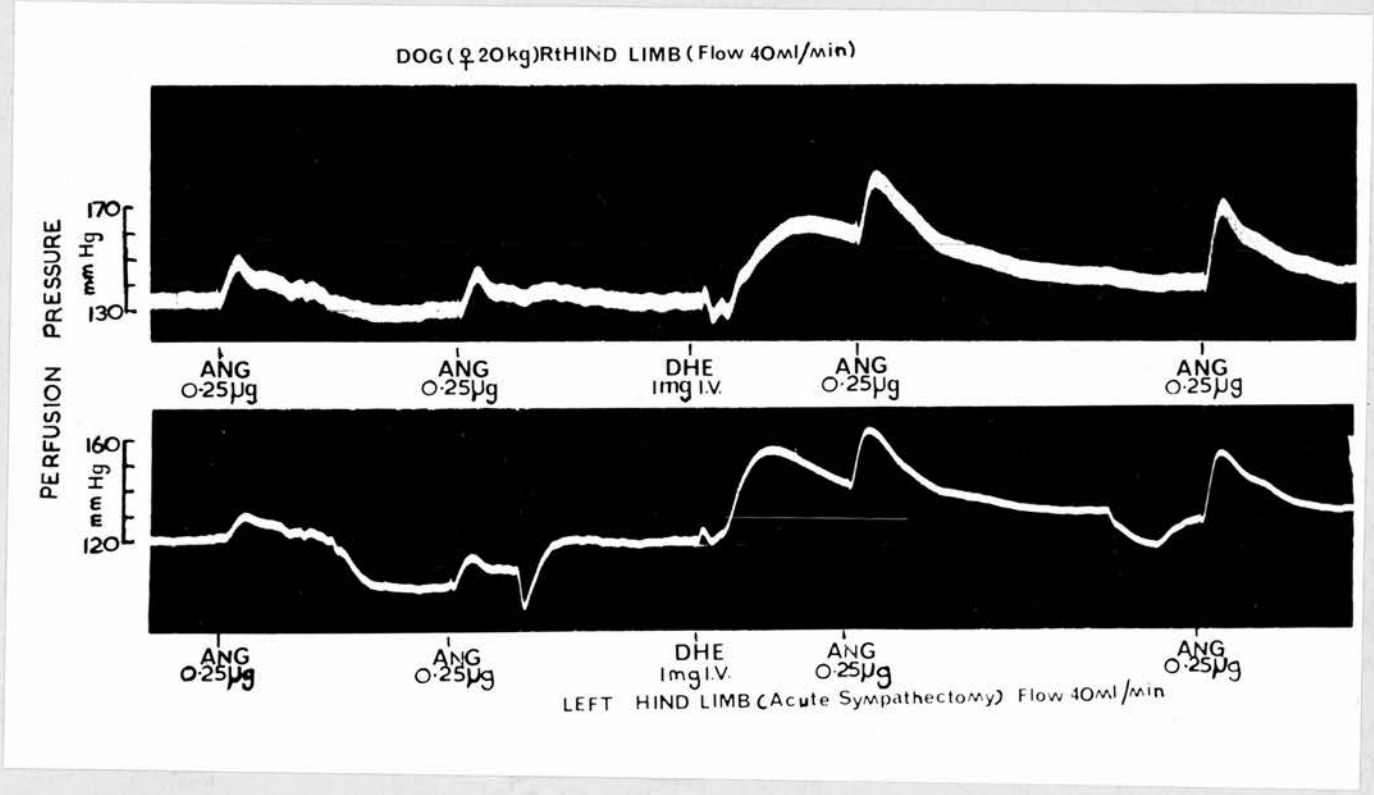


Figure 4

The effect of dihydroergotamine on the vasoconstrictor action of angiotensin in the hind limb of the dog perfused at constant flow.

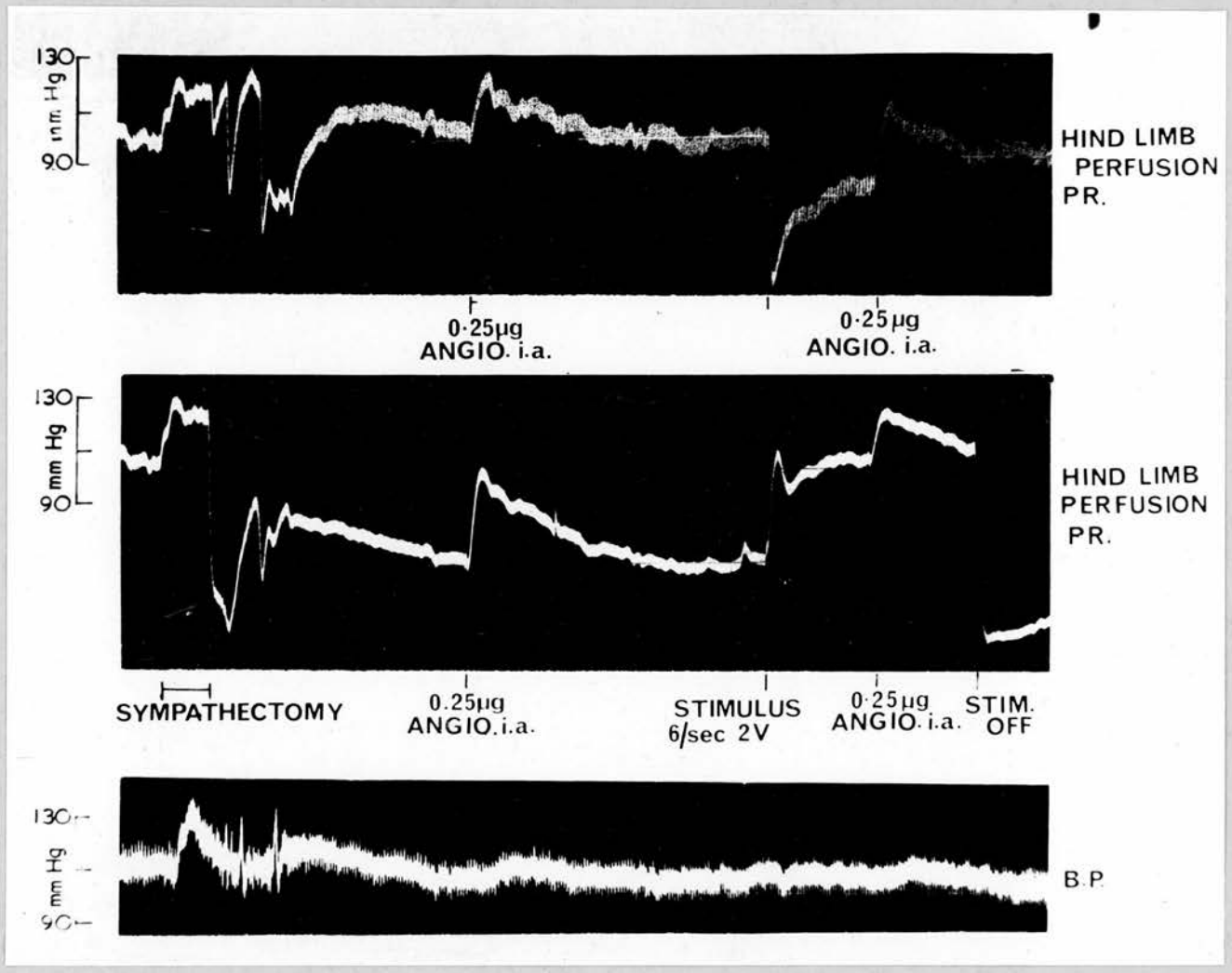


Figure 5

Effect of sympathectomy and stimulation of sympathetics on the vasoconstrictor action of angiotensin in the hind limb of the dog perfused at constant flow. (From experiment summarised in table 5 e)

TABLE 6

PROCEDURE	RAFFINOSE CONC. OF PLASMA IN ug/ml	PER CENT CHANGE RAFF. CONC.	PLASMA Sodium mEq/l	OBSERVED PER CENT CHANGE SODIUM	TRUE PER CENT CHANGE SODIUM
CONTROL	1.42		121.6		
3µg Angio I.A.	1.3	-8.5	124.0	+1.96	+10.5
PHENTOLAMINE 5mg I.A.	1.6	+13.4	118.0	-3.0	+10.4
3µg Angio I.A.	1.3	-17.4	119.0	+0.9	+18.3
3µg Angio I.A.	1.6	0	123.5	+4.7	+4.7
PRONETHANOL 25mg I.A.	1.82	-13.1	121.6	+3.1	+16.2
3µg Angio I.A.	1.8	-3.8	123.5	+1.6	+5.4
DIHYDROERGOT-AMINE 4mg I.A.	1.8	-3.3	121.0	-0.5	+2.8
3µg Angio I.A.	1.7	-2.3	124.0	+2.5	+4.8
CONTROL	4.4		116.0		
2µg Angio I.A.	4.7	+6.8	111.0	-4.3	-11.1
PHENTOLAMINE 2mg I.A.	4.2	-4.5	115.0	-0.9	+ 3.6
1µg Angio I.A.	4.4	+4.8	113.0	-1.7	+ 3.1
PRONETHANOL 7mg I.A.	3.9	-7.1	111.0	-3.5	- 3.6
1µg Angio I.A.	3.8	-2.6	113.0	+1.8	+ 4.4
CONTROL	6.8		119.0		
3µg Angio I.A.	6.8	0	115.0	-3.4	- 3.4
2µg Angio I.A.	6.6	-2.9	112.0	-5.9	- 3.0
PHENTOLAMINE 5mg I.A.	6.4	+5.9	118.0	-0.8	- 6.7
2µg Angio I.A.	6.4	0	110.0	-6.7	- 6.7
PRONETHANOL 25mg I.A.	6.5	+1.3	121.0	+2.5	+ 1.2
2µg Angio I.A.	6.5	0	110.0	-9.1	- 9.1

TABLE 6 (contd)

PROCEDURE	RAFFINOSE CONC. OF PLASMA IN ug/ml	PER CENT CHANGE RAFF. CONC.	PLASMA SODIUM mEq/l	OBSERVED PER CENT CHANGE SODIUM	TRUE PER CENT CHANGE SODIUM
CONTROL	5.2		132.0		
3µg Angio I.A.	5.2	0	139.0	+5.3	+5.3
2µg Angio I.A.	4.7	-9.6	141.0	+6.8	+16.4
PHENTOLAMINE 75mg I.A.	5.9	+13.4	141.0	+6.8	-6.6
2µg Angio I.A.	6.5	+10.2	139.0	-1.4	-11.6
PRONETHANOL 50mg I.A.	6.5	+10.2	134.0	-5.0	-15.2
2µg Angio I.A.	7.5	-15.4	134.0	0	+15.4
CONTROL	4.6		128.0		
1µg Angio I.A.	4.7	+2.1	126.0	-1.6	-3.7
CONTROL	4.7		130.0		
PHENTOLAMINE 2.5mg I.A.	5.7	+21.3	126.0	-3.1	-24.4
1µg Angio I.A.	3.3	-42.1	126.0	0	+42.1
CONTROL	3.2		134.0		
1µg Angio I.A.	4.1	+28.1	135.0	+0.7	-27.4
PHENTOLAMINE 2.5mg + PRONETHANOL 25mg I.A.	3.7	+15.6	131.0	-2.2	-17.8
DIHYDROERGOTAMINE 2mg I.A.	3.6	-2.7	125.0	-4.5	-1.8
1µg Angio I.A.	3.3	-8.3	126.0	-0.8	+7.5
1µg Angio I.A.	3.2	+11.1	128.0	+2.4	-8.7
CONTROL	2.2		138		
1µg Angio I.A.	1.8	-18.1	136.0	-1.4	+16.7
2µg Angio I.A.	1.8	-18.1	140.0	+1.5	+19.6
PRONETHANOL 30mg I.A.	2.0	-9.1	141.0	+2.2	-11.3
2µg Angio I.A.	1.8	-10.0	144.0	+2.1	+12.9
10µg Angio I.A.	1.4	+40.0	138.0	0	-40.0
PHENTOLAMINE 2.5mgI.A.	1.4	+40.0	136.0	-1.5	-41.5
2µg Angio I.A.	1.7	+21.4	136.0	0	-21.4
10µg Angio I.A.	1.1	+22.2	133.0	-1.5	-23.7

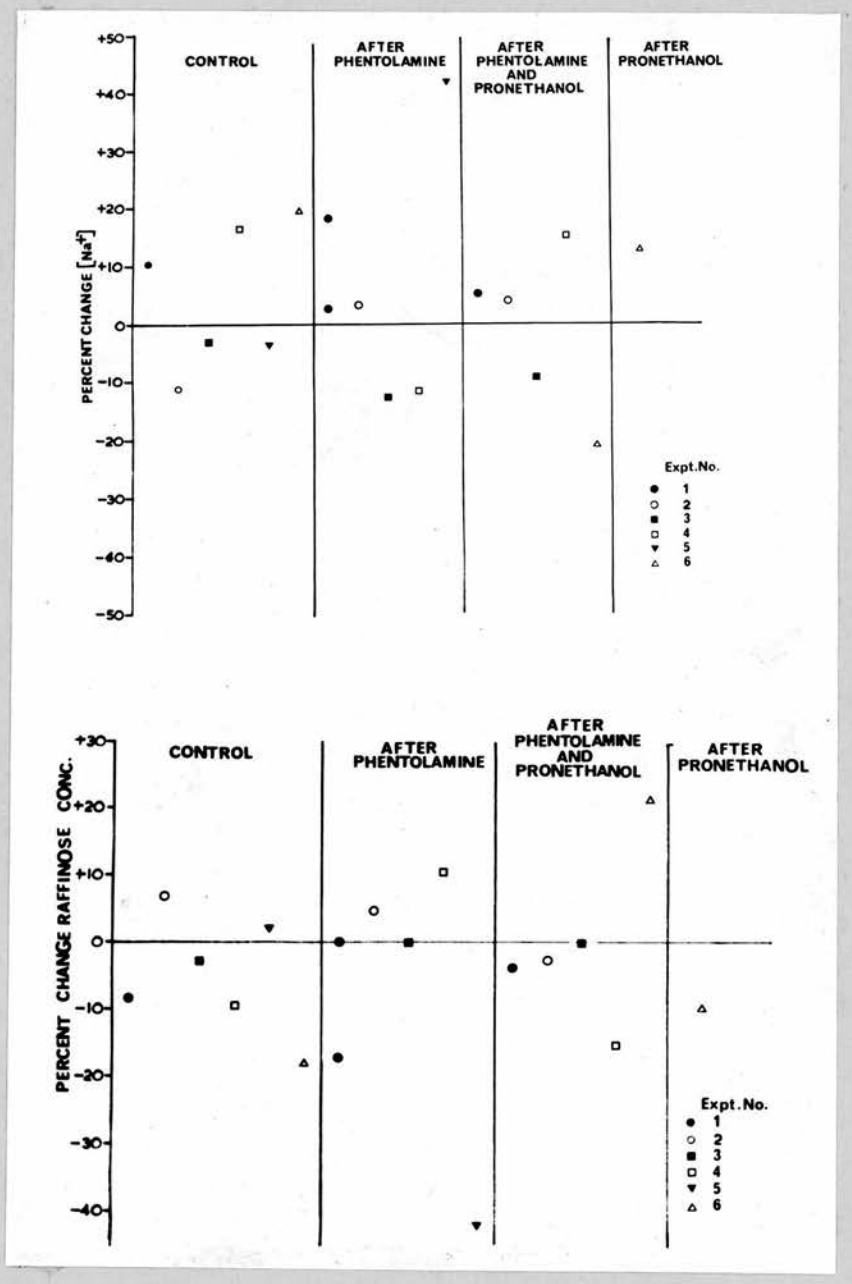


Figure 6

Sodium and water shifts measured in the venous blood from the hind limb of dogs following a single intra-arterial injection of angiotensin.

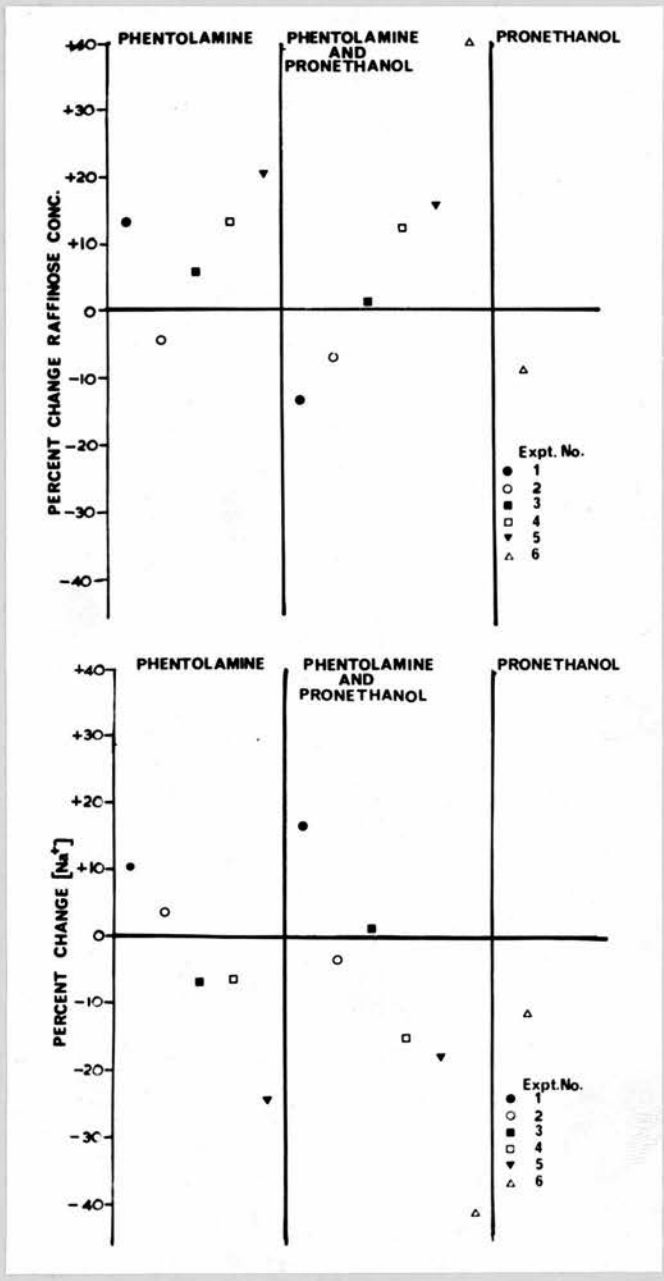


Figure 7

Sodium and water shifts in the venous blood from the hind limb of dogs following the intra-arterial administration of blocking agents.

CHAPTER 6

EXPERIMENTS USING ISOLATED BLOOD VESSELS

METHODS

(a) In these experiments arterial segments from rats were perfused with Krebs solution. The rats were anaesthetised with pentobarbitone and different main arteries were cannulated usually through the aorta while still in situ. The artery was then dissected free and severed distally so as to leave 5 to 10 mm attached to the cannula. The arterial segments were removed to a 5ml organ bath and perfused at a constant rate using a Sigmamotor pump. The Krebs solution was gassed with 95%O_2 & 5%CO_2 and had the following composition (mM/l) 142 Na^+; 5 K^+; 1.3Ca^{++} 1.3 Mg^{++};130 Cl^-; 30 HCO_3^- ; 1.3 $SO_4^=$; 1.2 $H_2PO_4^-$; 18 Glucose. The perfusate escaped from the cut end of the artery into the organ bath and was drawn off at the top so that the artery was totally immersed in the liquid in the bath. The temperature throughout was maintained at 37°C. The section of tubing between the pump and the arterial cannula was kept as short as possible and contained a 'T' junction from which the perfusion pressure of the artery was registered kymographically using a Condon mercury manometer. When the perfusion was commenced the tone of the arteries was high but within 15-20 mins the arterial segment relaxed and the rate of perfusion was then adjusted to give a resting baseline perfusion pressure of about 40--60mm of mercury. The base line pressure was well maintained in most preparation and the rate of perfusion was in the range of 4-6ml/min. In some experiments two fine platinum wires were wrapped round the artery over the section covering the cannula so that electrical stimulation could be applied. Vasoactive agents were administered into the tubing between the pump and the artery as either single injections when the

drug was dissolved in not more than 0·03ml of the perfusate to minimise the injection artefact on the pressure record or as continuous infusions using a Palmer infusion pump. 0.1mg ascorbic acid per ml was added to solutions of noradrenaline to prevent oxidation. To decrease the concentration of an ion in the perfusate it was replaced by an iso-osmotic amount of sucrose. Depolarising Krebs solution was prepared by replacing part of the NaCl by an osmotically equivalent amount of potassium sulphate so that the total potassium ion concentration was equal to 118mM/l. The calcium ion content of this solution was increased to 10mM/l to allow for ion pair formation.

In a few experiments chronically denervated renal arteries were used. Under ether anaesthesia the abdomen was opened and the adventitial coat was stripped off the renal artery close to its origin from the aorta. The stripping was done under a dissecting microscope. The abdomen was closed and 7 days later the animal killed and the renal artery cannulated and perfused as in the other experiments.

(b) In these experiments segments of human umbilical arteries were used. The umbilical cords were clamped at both ends and placed in cold Tyrode solution immediately after delivery and were used within half an hour. A six inch length of the cord free from knots was selected and one of the arteries cannulated at the foetal end. The piece of cord was then transferred to warm solution to relieve the spasm of the arteries. Warm solution was then pushed through the artery by means of a syringe to wash out any blood inside the artery, and the placental end of the artery was also cannulated. The piece of cord was then held vertically in an organ bath and perfused through the foetal end at a constant pressure head of about 70mm of mercury using a Mariotte bottle. The solution used had the following composition (g/L)NaCl 9.00; KCl 0.42; $CaCl_2$ 0.24; $NaHCO_3$ 0.2; Dextrose 1.0

was gassed with 90% O_2 and 5% CO_2 and maintained at 34°C (Gokhale, et al [redacted] 1966). The organ bath was also water jacketed and contained a small quantity of the solution surrounding the cord. The outflow from the placental end of the artery was led through the bottom of the organ bath to a Palmer drop counter recording on smoked paper. Drugs were injected into the perfusion stream close to the foetal end of the artery.

RESULTS & DISCUSSION

In studies on the effect of drugs on vascular smooth muscle the preparation used most frequently is the isolated rabbit aorta strip. This preparation is open to the objection that a large elastic vessel is used and the tension-length measurements are difficult to translate into meaningful pressure-radius parameters. The perfusion of isolated segments of artery have the advantage of maintaining the anatomical configuration of the muscle fibres permitting measurements of changes in the vessel tone in a manner which is more physiological. Burton & Stinson (1960) have shown that in such perfused segments of arteries under conditions of constant flow changes in the active tension of the smooth muscle cells of the vessel wall produce linearly related changes in the perfusion pressure.

The arterial stump can be made to constrict either by the injection of drugs into the perfusion stream or by stimulation of the nerves through the electrodes wrapped round the end of the artery. That such electrical stimulation produced a constriction via the perivascular nerves and not by stimulation of the muscle cells directly was shown by the finding that bretylium administration or withdrawal of calcium from the perfusate lead to abolition of the response, while injected noradrenaline still produced a contraction.

Several arteries from the rat (carotid, femoral, iliac, superior mesenteric & renal) were tested and some typical results are shown in Fig. 8. Noradrenaline produced a constriction in all of them and in a given artery after the first few doses a given dose of noradrenaline produced reproducible responses on repeated administration. The constrictor response to angiotensin was slower in onset and disappearance

when compared with the response to noradrenaline and all the arteries developed tachyphylaxis to angiotensin on repeated administration. The renal artery of the rat was however peculiar in not showing a constriction to angiotensin even on its first administration. As large a dose as 0.5 mg was found to be ineffective in the renal artery. This has been confirmed very recently by Hrdina, Bonaccorsi and Garattini (1967). Renal arteries from dogs and rabbits were also tested, and only those from rabbits were found to be responsive to angiotensin (fig. 9).

Although angiotensin by itself did not produce a constriction of the renal artery, it was noticed that subsequent responses to noradrenaline were potentiated for periods from 15 to 45 mins after the injection of angiotensin (Fig. 10). The potentiating effect could however be maintained by an infusion of angiotensin into the perfusion stream close to the arterial canmula. The concentration of angiotensin required to produce a potentiation varied from preparation to preparation but averaged 2.5μg/ml of perfusate. Some preparations were affected even at a dose of 0.01 μg/ml. The degree of potentiation was not dependent on the dose of angiotensin used but had an all or none character. The degree of potentiation was also variable from artery to artery. A typical dose response curve of a renal artery to single injections of noradrenaline before and after an infusion of angiotensin is shown in table 7 and figure 11 and it can be seen that the potentiation is effective over a large range of noradrenaline dose. A similar potentiating action is seen during continuous infusions of noradrenaline (fig.12).

Oxytocin vasopressin, tyramine, 5-hydroxytryptamine, adrenaline and nerve stimulation also produced constrictor responses in the renal artery, but of these only the responses to adrenaline, tyramine and nerve stimulation were potentiated by angiotensin (fig. 13). It may therefore be concluded that

the potentiating action of angiotensin was confined to the catecholamines and procedures that may be expected to release catecholamines, from tissue stores. In the other arteries that were tested angiotensin did not show this potentiating effect consistently and when it did occur it was only to a slight extent. The lack of an effect of angiotensin on the response of the superior mesenteric artery of the rat to noradrenaline has also been reported by McGregor (1965).

To get more information about the mechanism by which angiotensin potentiates the response of the renal artery to catecholamines, the effect of cocaine on this effect was investigated. Cocaine was selected as it seems to act fairly selectively on adrenergic transmission. There is little evidence that cocaine has any marked effects on noradrenaline storage or metabolism in sympathetic nerves while there is good evidence that it acts by potently inhibiting the re-uptake of noradrenaline into the nerve terminals. Figure 14 shows that the addition of cocaine to the perfusate increases the response to noradrenaline as expected but the subsequent addition of angiotensin further increases the response. Iversen (1961) has shown that in the isolated heart at the concentration of cocaine used in this experiment (10^{-4}M) 100% inhibition of the noradrenaline uptake process occurs at an external noradrenaline concentration of 200ng/ml. Therefore the uptake process may be expected to be largely if not completely inactivated in the artery preparation. The fact that angiotensin is still effective then suggests that its action does not involve a mechanism of inhibition of noradrenaline uptake. Vessels denervated 7 days previously also showed a sensitisation of the response to noradrenaline when angiotensin was infused further proving that the nerves were not directly concerned.

The influence of changes in the external ionic composition was also tried. In these experiments a single dose of noradrenaline was used to test the response of the renal artery stump throughout. After the artery

had been perfused with Krebs solution lacking a particular ion for about 30 min at least three responses to the selected dose of noradrenaline was recorded. Angiotensin was then infused and the responses to the same dose of noradrenaline recorded again. The mean response before and after angiotensin infusion was calculated and the significance of the difference between them in several arteries tested using the 't' test, table 8. In a control group of 5 arteries where normal Krebs solution was used as the perfusate a greater response (54.5% average) to noradrenaline was observed after angiotensin significant at the 0.01 level. When the sodium in the perfusate was reduced 50% by substitution with sucrose the difference in the response produced by angiotensin still reached significance at the 0.01 level. With a complete withdrawal of the calcium or potassium or the magnesium from the perfusate the difference produced by angiotensin was not significant. Elevation in the external potassium to a concentration of 118mM/l can be expected to depolarise the cell membrane. This is usually accompanied by an increase in the tension of the artery but it retains its responsiveness to noradrenaline. Under these conditions however the effect of angiotensin on the response to noradrenaline is completely abolished (fig.15). An increase in the calcium in the perfusate to 10mM/l however greatly increased ($P< 0.001$) the potentiating action of angiotensin on the response of the renal artery to noradrenaline.

The effect on the smooth muscle cell of changes in the external ionic composition is bound to be complex as each ion probably acts at more than one site, and not all of these actions are at present fully understood. Casteels & Kuriyama (1966) have shown that at least in the guinea-pig taenia coli the potassium equilibrium potential is the main factor in the generation of the membrane potential. Bulbring & Kuriyama (1963) have

studied the effect of changes in the external concentration of calcium and sodium on the same tissue and shown that an increase in the external calcium leads to an increase in the membrane potential and vice versa. Also replacement of 50% of the external sodium by Tris produced a slight hyper-polarisation of the membrane. In the absence of similar studies on vascular smooth muscle the assumption has to be made that this tissue behaves in a similar way, fully realising the possibility that this may prove not to be the case. If vascular smooth muscle behaves like guinea-pig taenia coli an elevation of the external K^+ concentration to 118mM/l would greatly reduce or abolish the resting membrane potential. Removal of calcium ions from the perfusion medium can also be expected to reduce the resting membrane potential moderately as the interstitial calcium concentration would also be reduced though there is evidence that the interstitial concentration may not be reduced to zero due probably to some sort of calcium binding. Both these changes in external ion composition were observed to greatly diminish or abolish the influence of angiotensin on the noradrenaline response, suggesting that this action is linked to the membrane potential or a function dependent on it. On the other hand when the external potassium ion concentration is lowered, a change that would lead to hyper-polarisation of the membrane, the angiotensin effect is once again attenuated. Another procedure which by analogy with taenia coli should lead to a hyper-polarisation of the membrane, viz. increase in the external calcium concentration, however does not decrease but greatly enhances the angiotensin effect. So that in so far as these two ionic changes lead to the same effect on the resting membrane potential their influence on the angiotensin effect is not consistent. If the influence of the ionic changes that lead to a depolarisation of the membrane is looked at again it is clear that a reduction in the calcium has almost as big an

influence as the increase of the potassium even though the former can be expected to produce a smaller degree of depolarisation. The results therefore point to the involvement of calcium in the sensitising action of angiotensin. A possible mechanism is that angiotensin exerts a permissive role in the entry of calcium through a polarised membrane. Waugh (1962 b) has shown that adrenaline augments calcium induced contractions of calcium depleted blood vessels in both normal and high potassium media, and has proposed that the adrenergic neurohumour causes vasoconstriction by a membrane action which is basically non electrical and which increases the availability of calcium ions to the contractile protein in the cell. It is known also that depolarised smooth muscle preparations allow greater freedom for the entry of external calcium suggesting that a charged membrane forms some sort of barrier. If angiotensin played a part in helping the entry of calcium through a charged membrane it would account for the observation that high potassium and low calcium media decreased its sensitising action on the response to noradrenaline. Similarly an isolated elevation of the membrane potential by decrease in the external potassium inhibits the sensitising action of angiotensin as penetration of calcium is now even more difficult. This adverse effect of hyperpolarised membrane can however be offset by an elevation in external calcium concentration, thereby increasing the concentration gradient tending to drive the calcium into the the cell when angiotensin is exerting its permissive role in promoting calcium entry.

In this context it is of interest that another drug (serotonin) which also sensitises the action of noradrenaline on isolated blood vessels also loses its effect in high potassium solutions (de la Lande, Connell & Waterson 1966). Though the mechanism of this sensitising action of serotonin is not definitely known it has been shown by Woolley (1958) that serotonin

plays a role in calcium transport across the membrane in the rat uterus, and that it can for instance restore the response of this tissue to acetylcholine - a drug which is itself dependent on calcium ions for its action - after this has been abolished by the calcium chelating agent Versene. It is therefore probable that the sensitising action of serotonin on the noradrenaline response is mediated through its calcium transporting action. If this is the case a close parallelism would exist between the action of serotonin and angiotensin on vascular smooth muscle.

An alternative interpretation of the sensitising action of angiotensin on the noradrenaline response of the renal artery may be that it is a manifestation of the established ganglion stimulating effect of angiotensin. This would be possible if extra-ganglionic synaptic sites existed along the course of the renal arteries. By stimulating these cells angiotensin would tend to increase the concentration of noradrenaline in the vicinity of the receptor and thereby increase the response to exogenous noradrenaline. The effects of the changes in ionic composition of the perfusion medium would then be due to their influence on the nerve endings. The results of the experiments where denervation was attempted are not necessarily contradictory to this interpretation as it would be difficult to remove such synaptic sites completely by stripping alone. Against this interpretation, however, is the finding that in a bretylium treated artery angiotensin is still effective in sensitising the response to injected noradrenaline (fig.16)

As pointed out earlier the renal artery is constricted by fairly large doses of oxytoxin. Just as with angiotensin such a single injection of oxytocin potentiated the responses to subsequent doses of noradrenaline. A continuous infusion of a sub-constrictor dose of oxytocin could maintain this potentiating effect as in the case of an angiotensin infusion (fig 17)

Vasopressin however was ineffective in influencing the noradrenaline response.

It was decided to use umbilical arteries as these have been reported to be devoid of an innervation (Von Euler 1938). A constant flow perfusion system did not work satisfactorily in this preparation as noradrenaline failed to induce very powerful contractions, and the tissue appeared to deteriorate rapidly under these conditions. The constant pressure perfusion technique was therefore adopted in these vessels. Under these conditions however induced changes in flow are not directly proportional to changes in the active tension developed by the smooth muscle. There is also the additional difficulty that a constrictor drug by reducing the flow would tend to remain in contact with the tissue for a longer length of time which cannot be controlled.

In some of these preparations noradrenaline produced a constriction while angiotensin was ineffective in all. On two occasions an infusion of angiotensin was observed to produce a potentiation to the response to noradrenaline (fig 18) as in the renal artery of the rat. More data will however be required on this point.

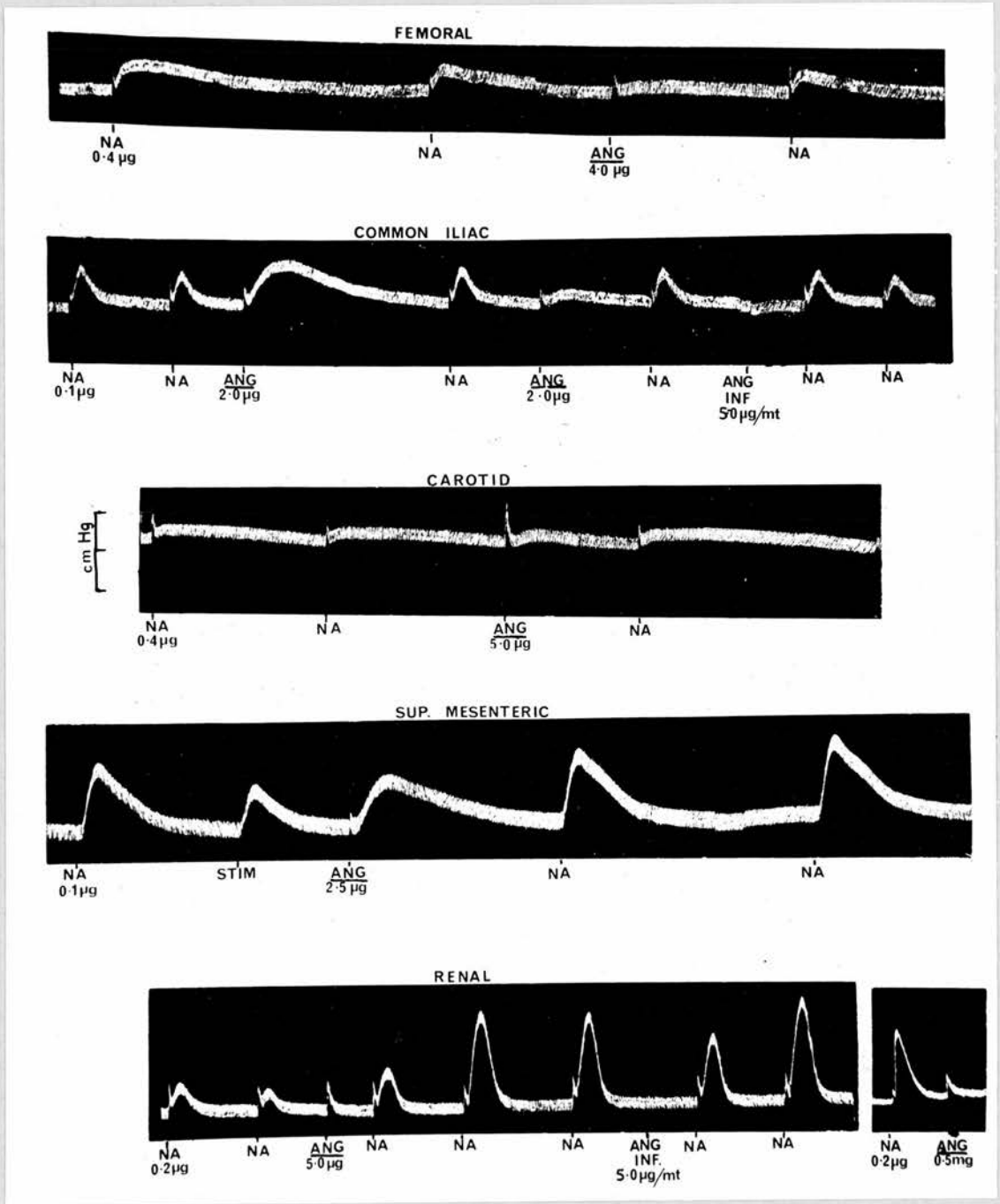


Figure 8

Effect of noradrenaline and angiotensin on different isolated perfused arterial segments of the rat. Showing lack of response to angiotensin in the renal artery.

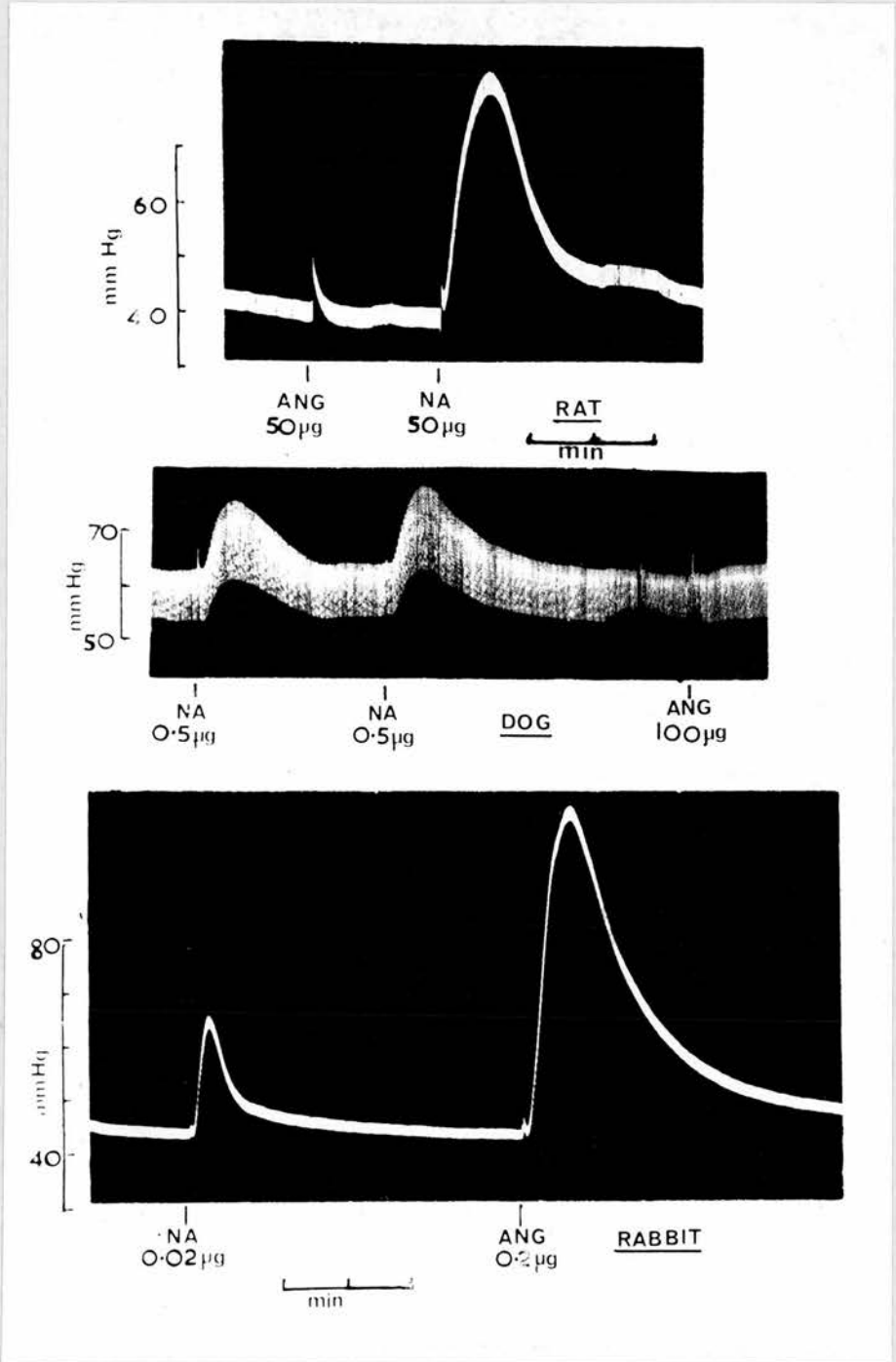


Figure 9

Effect of angiotensin on renal arterial segments from a dog, rabbit and rat.

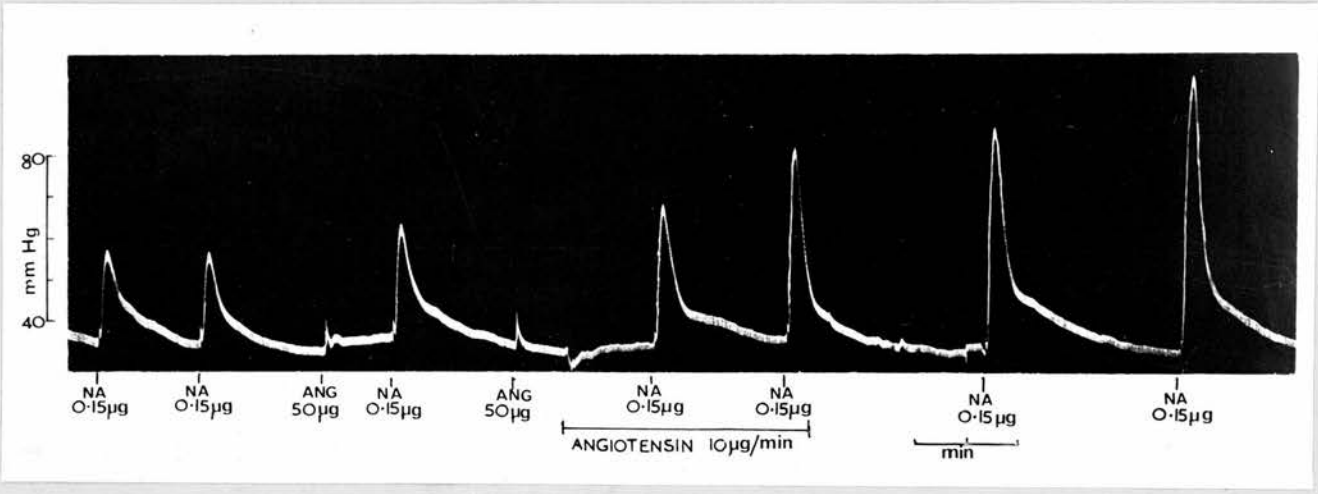


Figure 10

Effect of a short infusion of angiotensin on the response of the renal artery to single injections of noradrenaline.

Table 7

DOSE OF ANGIOTENSIN IN NANOGRAMS	CHANGE IN PERFUSION PR. MM. Hg.	MEAN	STD DEV
	BEFORE ANGIOTENSIN		
100	20.0; 22.0; 23.5;	21.8	1.7
320	49.0; 51.0;	50.0	1.0
560	74.0; 82.0;	78.0	4.0
1000	98.5; 98.5;	98.5	-
	AFTER ANGIOTENSIN 1µg/ml		
100	44.0; 47.0;	45.5	2.2
320	88.0; 89.5;	88.8	0.7
560	105.5; 113.0;	109.3	3.7
1000	123.5; 130.0;	126.8	3.2

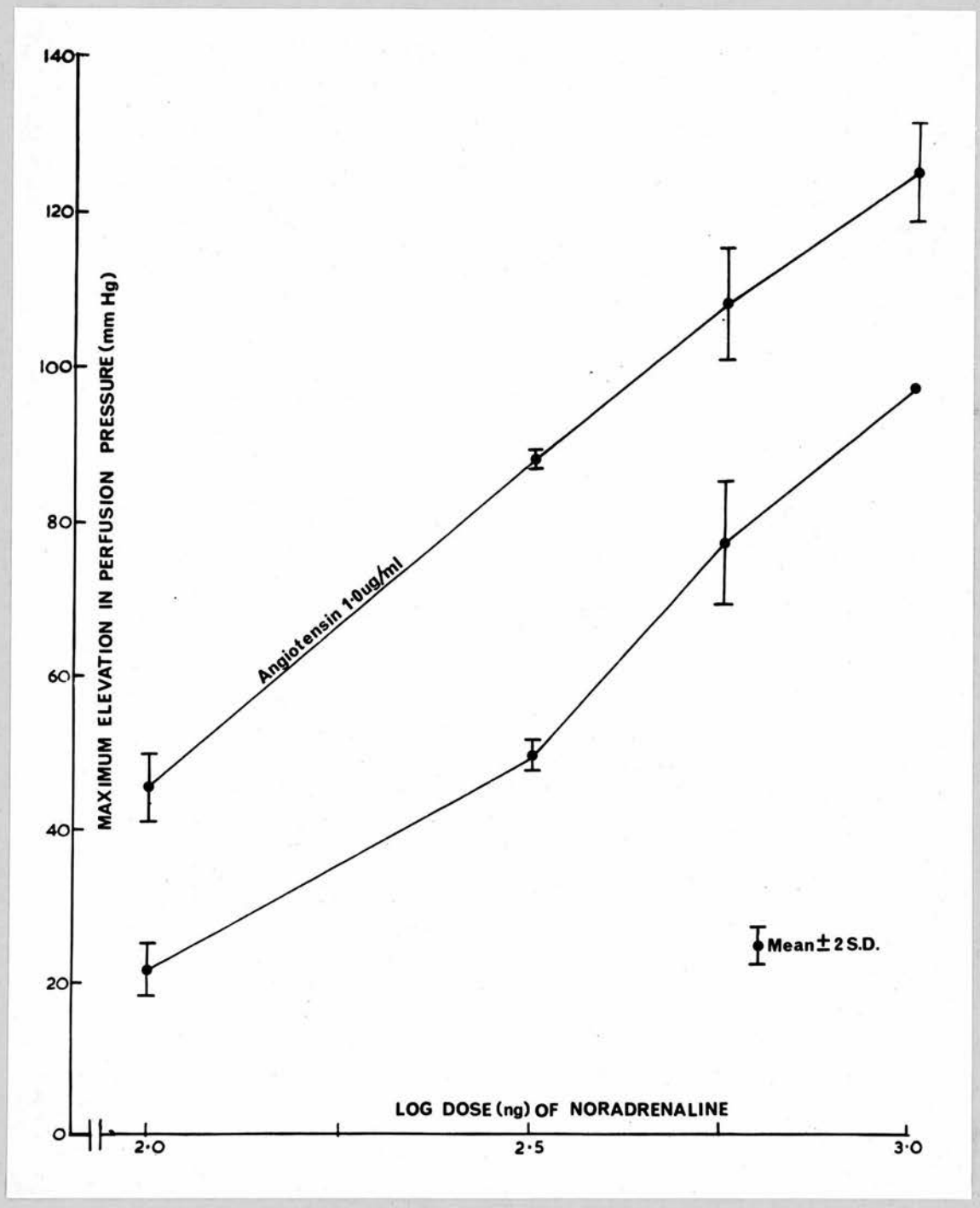


Figure 11

Dose-response relationship of a rat renal artery to single injections of noradrenaline before and during an infusion of angiotensin.

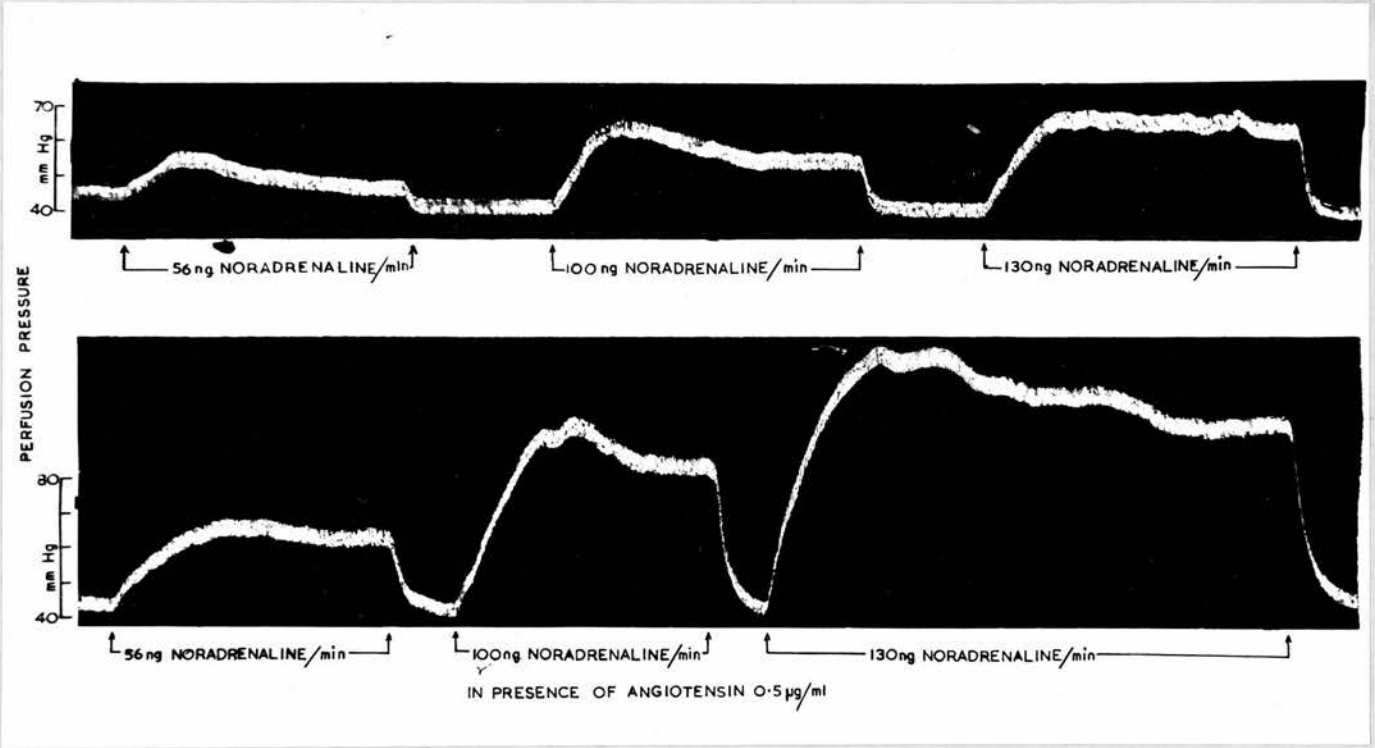


Figure 12

Effect of angiotensin on the constrictor responses in the renal artery due to continuous infusion of different amounts of noradrenaline.

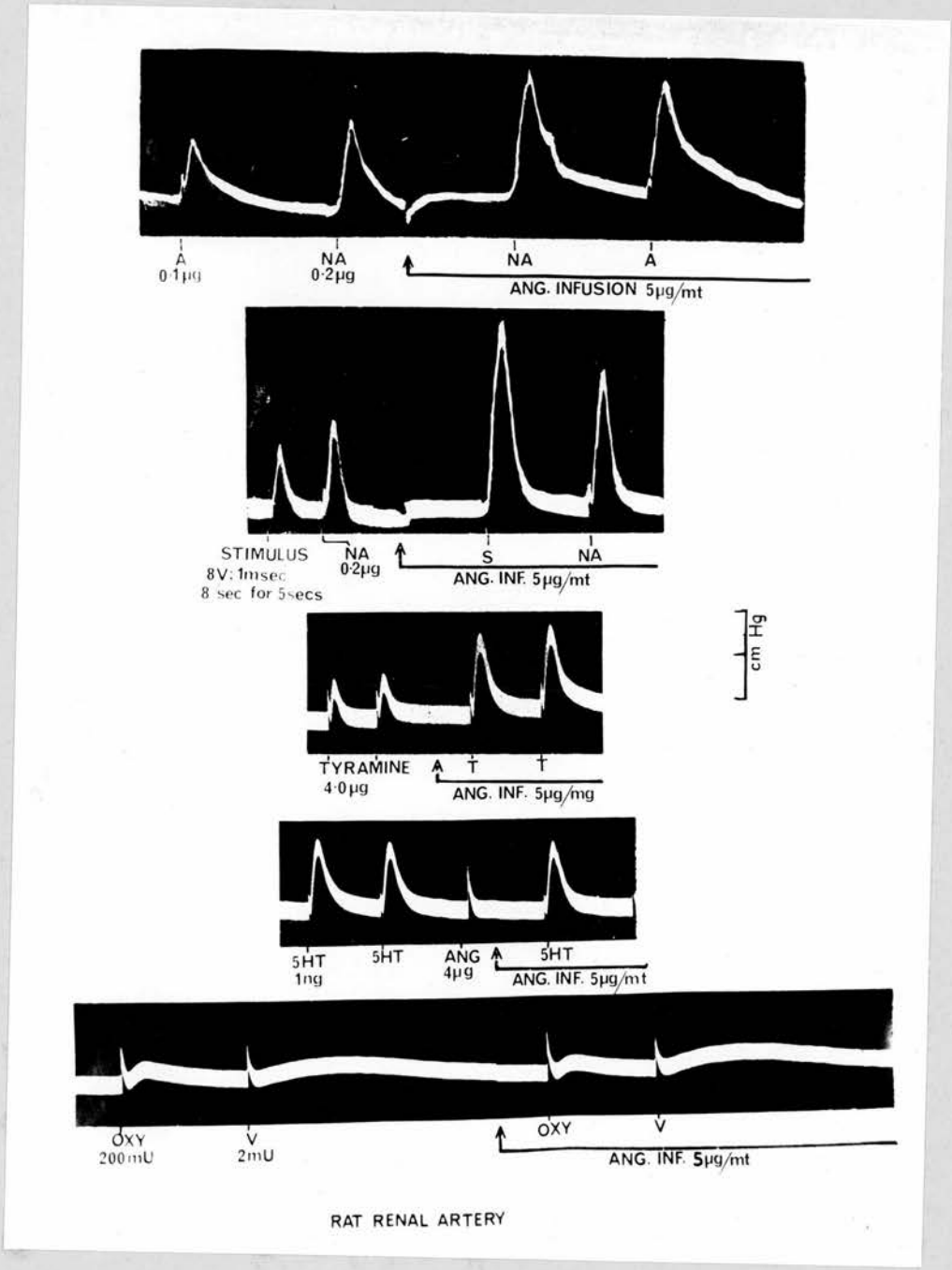


Figure 13

Effect of angiotensin on the constrictor response of the renal artery to several drugs and nerve stimulation.

A - Adrenaline
NA - Noradrenaline
V - Vasopressin
O - Oxytocin
T - Tyramine
5 - HT - 5-Hydroxytryptamine

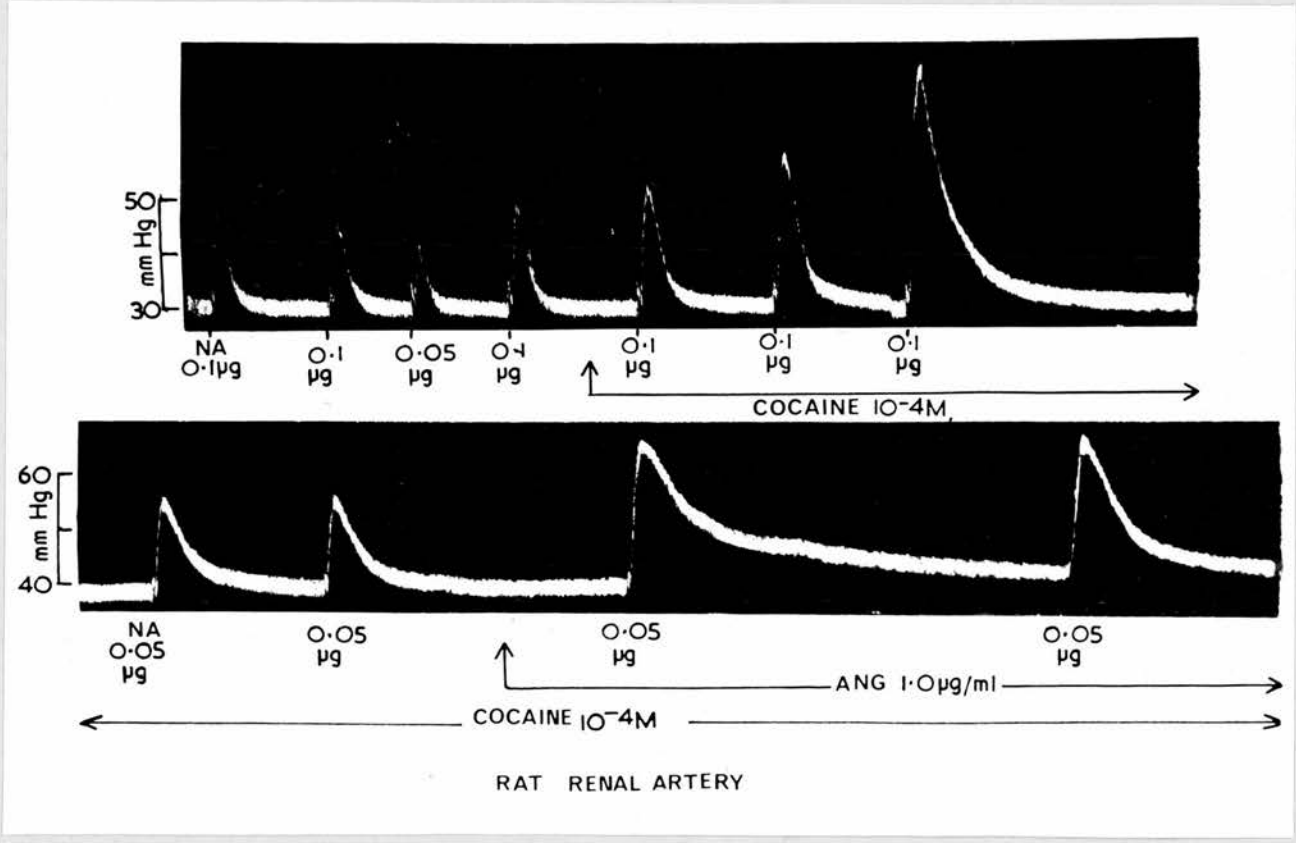


Figure 14

The sensitising action of cocaine and angiotensin on the noradrenaline response in the rat renal artery.

Table 8

RESPONSES BEFORE ANGIOTENSIN MM. Hg.	MEAN	RESPONSES AFTER ANGIOTENSIN MM. Hg.	MEAN	DIFF. OF MEANS	PER CENT CHANGE
NORMAL KREBS (controls)					
13.0; 17.5; 26.0; 21.0; 25.5;	20.6	28.0; 24.5; 24.0; 28.0	26.1	5.5	+26.7
25.0; 27.0; 30.0;	27.3	45.0; 39.0; 35.0;	39.7	12.4	+45.4
12.0; 11.0; 11.0;	11.3	21.0; 20.0; 18.5;	19.8	8.5	+75.2
16.0; 16.0; 16.0; 18.0;	16.5	30.0; 32.0; 33.0; 34.0;	32.3	15.8	+95.7
12.0; 12.0; 14.0; 15.0;	13.3	25.0; 33.0; 35.0;	31.0	17.7	+27.8
P< 0.01			MEAN PER CENT CHANGE		+54.2
Na^+ REDUCED 50%					
28.0; 29.0; 31.0;	29.3	39.0; 39.0; 45.0;	41.0	11.7	+39.9
39.0; 32.0; 39.0;	36.7	47.0; 40.0; 40.0;	42.3	5.6	+15.2
20.0; 20.0; 18.5;	19.5	22.0; 19.0; 19.0;	20.0	0.5	+ 2.5
16.0; 19.0; 21.0;	18.7	24.5; 22.0; 22.0;	22.8	4.1	+21.9
33.0; 29.0; 32.0;	31.3	34.0; 34.0; 33.0;	33.7	2.4	+ 7.7
33.0; 35.0; 35.0;	34.3	42.0; 39.0; 38.0;	39.7	5.4	+ 15.7
P = 0.01			MEAN PER CENT CHANGE		+17.2
K^+ FREE KREBS					
21.5; 24.0; 25.0;	23.5	23.0; 24.5; 25.5 24.0;	24.3	0.8	+ 3.4
17.0; 17.0; 19.0;	17.7	18.0; 20.5; 20.0;	19.5	1.8	+10.1
11.0; 12.5; 12.5;	12.0	13.0; 12.5; 13.0;	12.8	0.8	+ 6.6
P > 0.05			MEAN PER CENT CHANGE		+ 6.7

Table 8 (contd)

RESPONSES BEFORE ANGIOTENSIN MM,Hg.	MEAN	RESPONSES AFTER ANGIOTENSIN MM,Hg,	MEAN	DIFF OF MEANS	PER CENT CHANGE
Ca^{++} FREE KREBS					
12.5; 10.5; 10.0;	11.0	11.0; 9.0; 10.0;	9.0	2.0	-18.2
20.0; 19.0; 19.0;	19.3	19.0; 22.0; 20.0;	20.3	1.0	+ 5.1
6.0; 6.0; 5.0;	5.7	7.0; 6.0; 5.5;	6.2	0.5	+ 8.8
P > 0.05			MEAN PER CENT CHANGE		+ 1.4
Mg^{++} FREE KREBS					
41.0; 38.0; 39.0;	39.3;	42.5; 46.0;	44.3	5.0	+12.7
26.0; 26.0; 32.0;	28.0	39.0; 27.0; 38.0;	38.0	10.0	+35.7
20.0; 22.0;	21.0	31.0; 21.0; 22.0;	24.7	3.7	+17.6
P > 0.05			MEAN PER CENT CHANGE		+22.0
INCREASED Ca^{++}(10mM/1) KREBS					
23.5; 25.0; 26.5;	25.0	36.0; 35.0; 35.0;	35.3	10.3	+41.2
5.0; 2.0; 2.0;	3.0	6.0; 6.5; 6.0;	6.2	3.2	+106.7
3.0; 3.5; 5.0;	3.8	8.0; 9.0; 7.0;	8.0	4.2	+110.5
P < 0.001			MEAN PER CENT CHANGE		+ 86.1

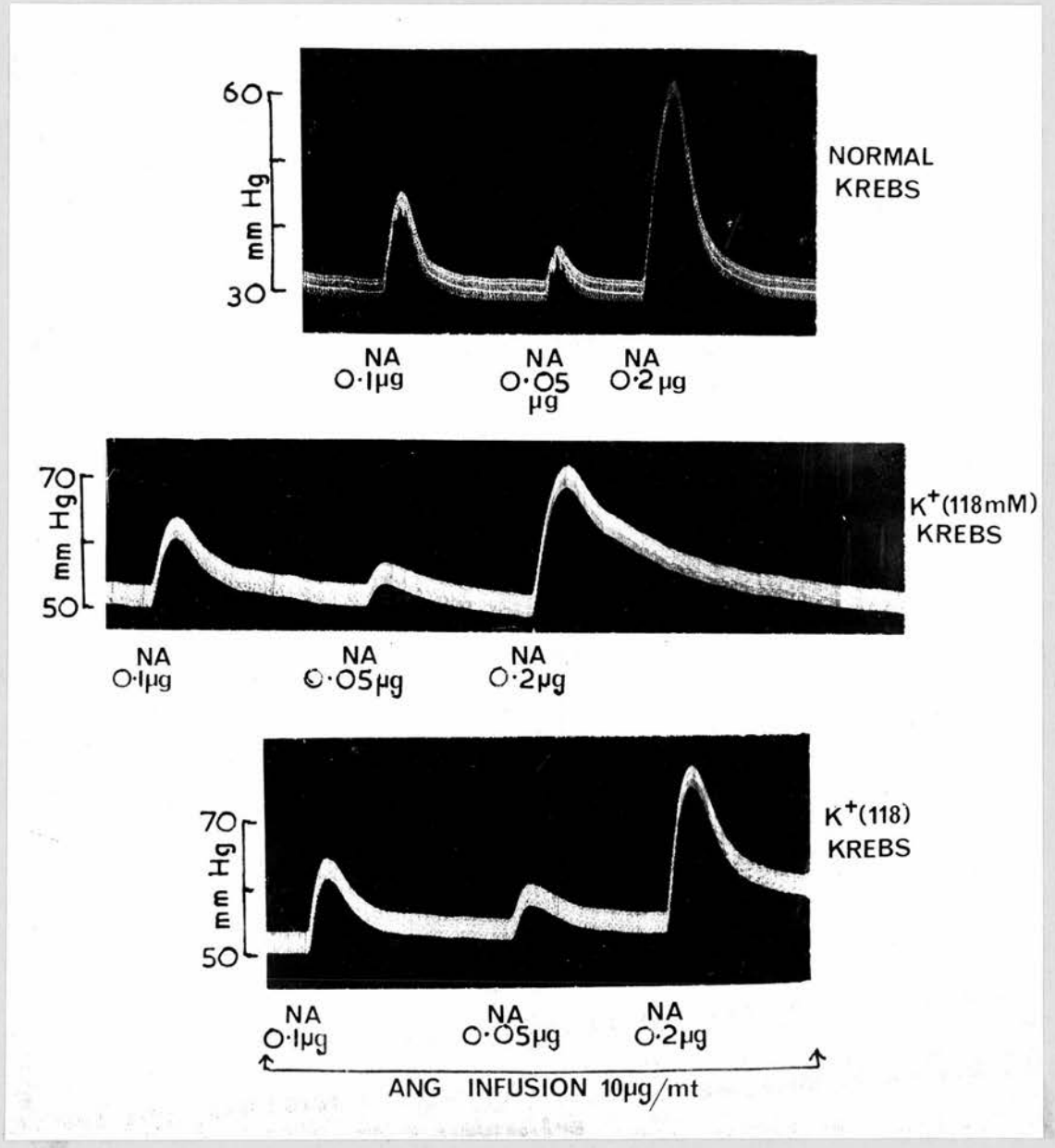


Figure 15

Effect of angiotensin on the noradrenaline response in a renal artery perfused with depolarising Krebs solution.

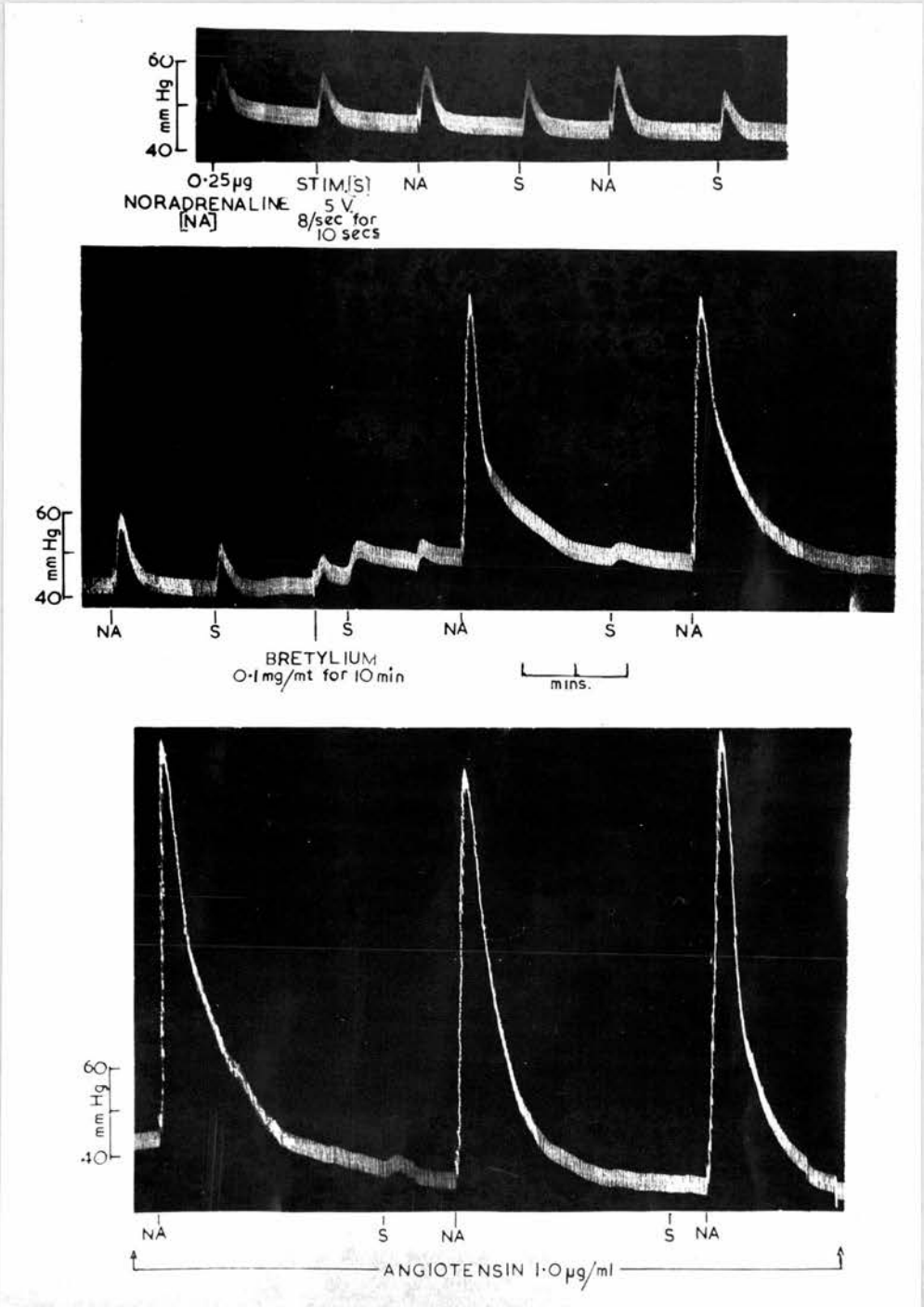
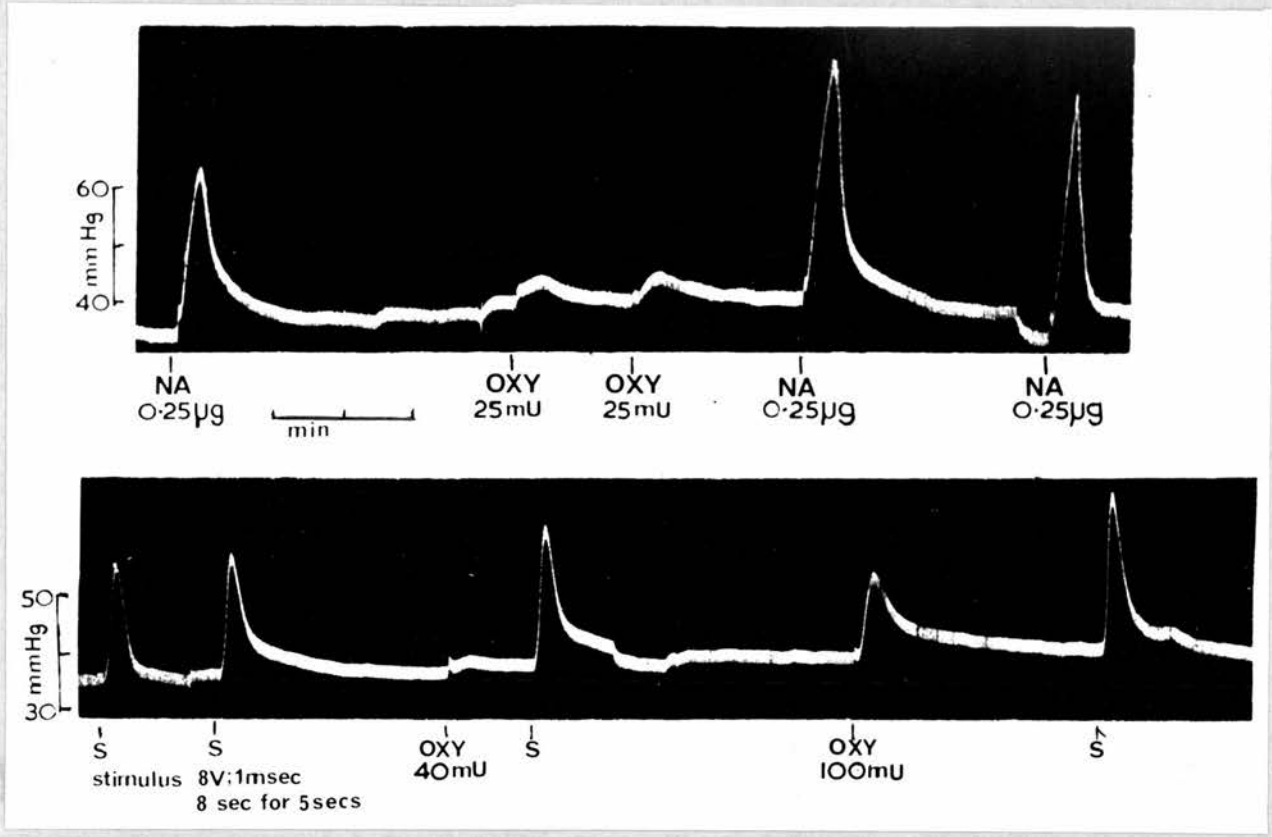


Figure 16

The sensitising action of angiotensin in the presence of bretylium.



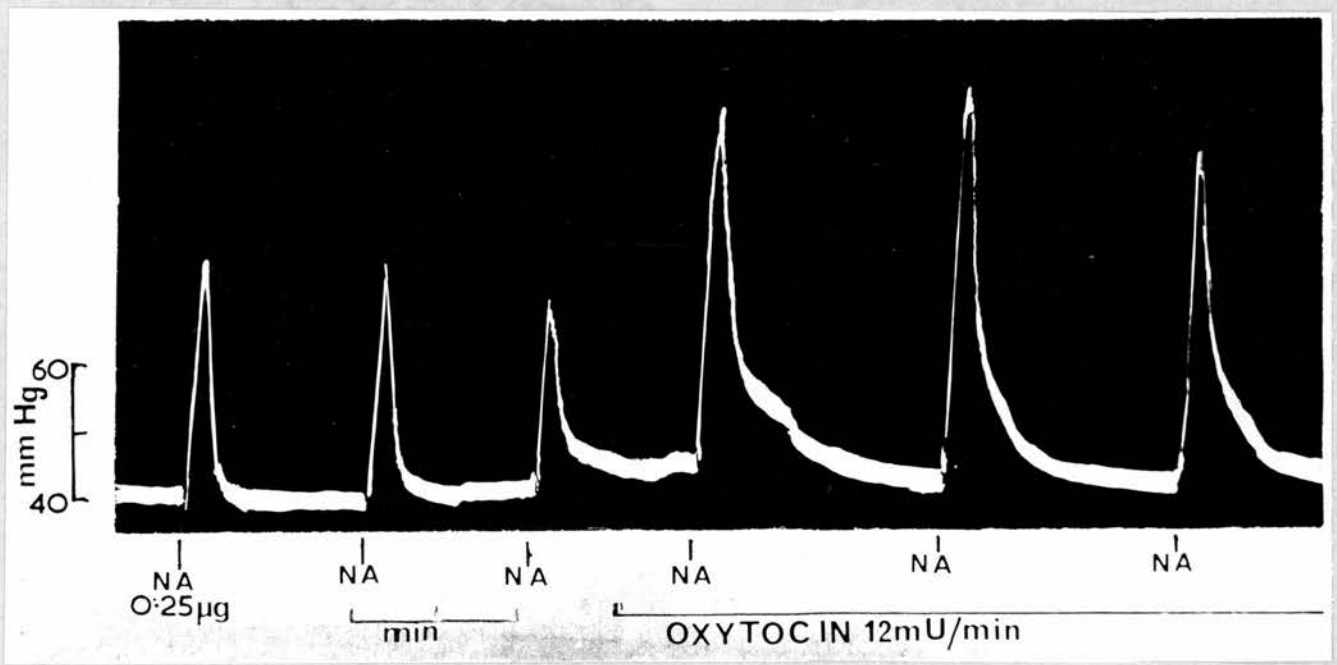


Figure 17

Effect of oxytocin on the noradrenaline response of the rat renal artery.

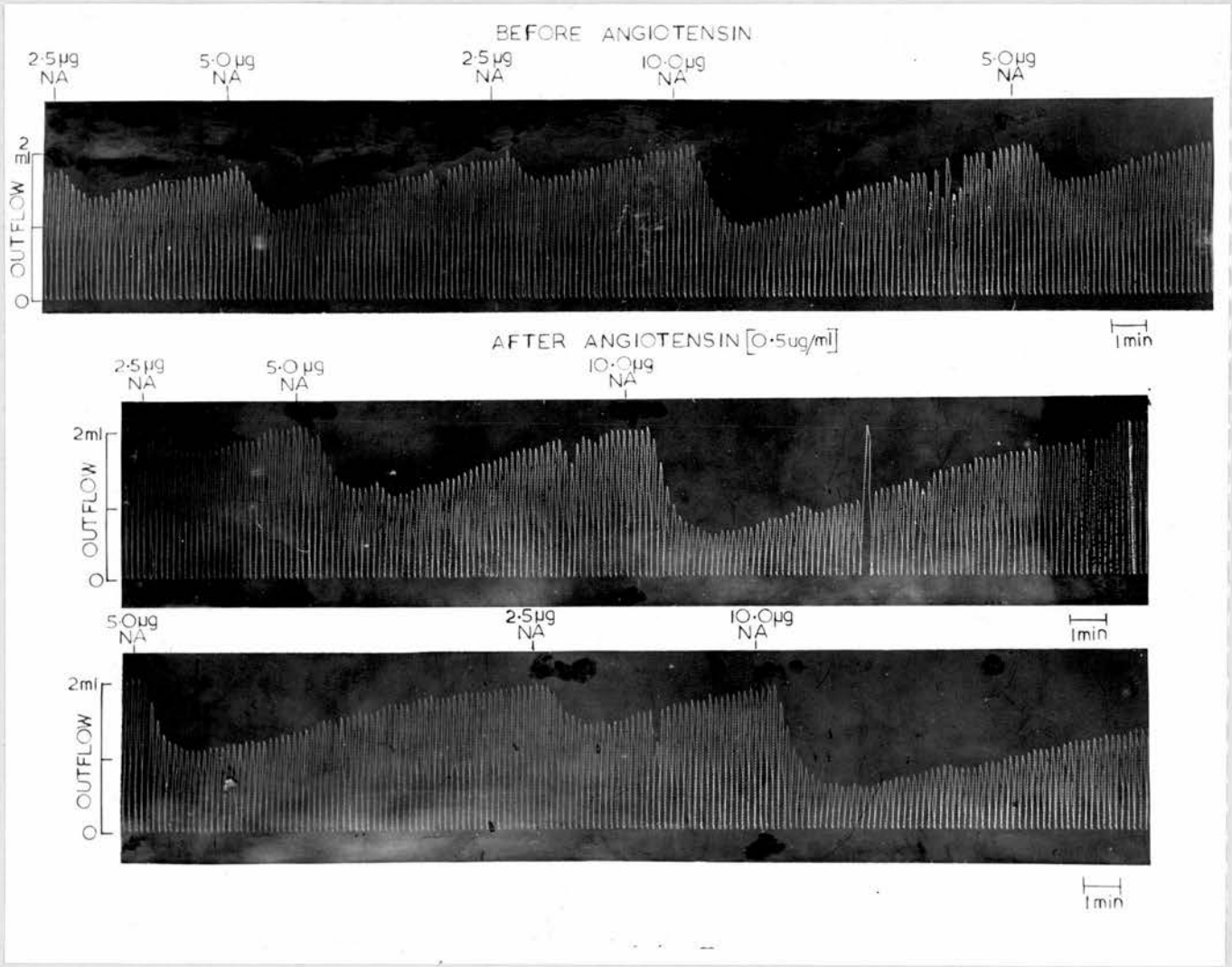


Figure 18

Effect of angiotensin on the response to noradrenaline in a human umbilical artery.

CHAPTER 7

SUMMARY OF CONCLUSIONS

The experiments embodied in this work closely followed those done earlier with oxytocin. Angiotensin being a potent vasoconstrictor was not expected to give a clear cut reversal in the vascular response as observed with oxytocin following interference with the sympathetics or administration of oestrogen. Statistical treatment of the pressor responses induced in rats showed no significant influence of oestrogens on the response to angiotensin, although animals in natural oestrus gave bigger responses than animals in other stages of the reproductive cycle. Pregnancy and pseudo-pregnancy were observed to depress the pressor response to angiotensin. Evidence that this is probably due to the presence of progesterone was found, and further it would appear that this is not a consequence of any secondary effect of an increased level of progesterone. These findings are clearly different from those observed with oxytocin and vasopressin whose constrictor activities are enhanced by oestrogen and progesterone.

Drugs interfering with sympathetic transmission did not produce a consistent effect as with oxytocin. Reserpine was found to markedly potentiate the pressor action of angiotensin. Certain other drugs which had the common feature of increasing the response to noradrenaline were also found to affect the response to angiotensin in the same direction as reserpine, suggesting that part of the pressor action of angiotensin was mediated through processes involving the adrenergic neurohumour.

Indirect though the evidence may be the effect of changes in the extracellular ion concentration suggested that electrical changes at the cell membrane contributed to the constrictor response elicited by angiotensin.

The experiments with dogs were not in agreement with those on rats. Thus there was no evidence for an influence of angiotensin on physiological processes occurring through the sympathetic nerves. The studies on ionic shifts induced by angiotensin appear to contradict the findings of Friedman et al.

The experiments using isolated arteries have shown an interesting additional effect of angiotensin in the rat renal artery. Here, though angiotensin had no constrictor action, it sensitised the preparation to the action of catecholamines. The effect of drugs and extracellular ion concentration on this effect of angiotensin is interpreted to mean that angiotensin plays a permissive role in the entry of calcium into the vascular smooth muscle cell.

ACKNOWLEDGEMENTS

I wish here to put on record my gratitude to Professor Whitteridge F.R.S. for not only affording me facilities in his department for undertaking research but also for extending to me the privilege of serving on the staff in a temporary capacity in the last year of my stay.

It is with the deepest gratitude that I acknowledge my indebtedness to Professor Mary Pickford F.R.S. To her I owe whatever expertise of experimental research I possess, and her sustained interest in the work and encouragement when things looked utterly black have been vital for its continuance. All experiments using dogs were done in conjunction with her. This acknowledgement would be incomplete if I were not to mention her personal kindness and understanding which has sustained me through out my stay and to me the association has been an education in the fullest sense of the word.

My sincere thanks also to Sybil Lloyd B.Sc. M.Sc. for many a helpful discussion and suggestion. All the dog experiments using the sodium electrode were done in conjunction with her. My thanks are due also to Dr. A. D. Dewar, B.Sc., Ph.D. and Dr. H. Begbie, M.A., Ph.D., M.B., Ch.B. for helpful discussions of matters arising from this work.

To Sister Staples and her staff at the labour room of the Simpson Memorial Hospital I am grateful for freely providing me with umbilical cords in spite of the busy routine at the ward. I am grateful also to the technical staff at the Department of Physiology, in particular to Mr T. May, chief technician, Mr J. Robertson, who performed most of the sodium estimations, and Mr Lawson, who is responsible for the photographic reproductions.

Finally a word of thanks to my wife who was helpful throughout the compilation of this thesis and for bearing stoically many a dull evening all for the sake of science.

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